444. *Investigations on the Biosynthesis of Morphine Alkaloids* *

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The conversion of a quintuply labelled reticuline into thebaine in the opium poppy has proved that demethylation of reticuline does not precede its further transformation in the plant into the morphine skeleton. The partial synthesis of the postulated initial dienone cyclisation product of reticuline has been accomplished from thebaine. This dienone is identical with the natural alkaloid salutaridine isolated by Professor R. A. Barnes. Salutaridine has been reduced to two epimeric salutaridinols. By appropriate tritium labelling the incorporation of salutaridine and of the salutaridinols into thebaine and morphine has been studied. Salutaridine and one of the salutaridinols are transformed by the plant into morphine alkaloids in remarkably high yield and without scrambling of the labels. Several of the final stages of the biosynthesis of morphine alkaloids are thus clearly defined and in accord with earlier hypotheses. Part of this work has been reported in preliminary form.[1-7]

The biosynthesis of the morphine (IV; R = H) alkaloids by oxidative cyclisation of a benzylisoquinoline precursor, itself derived from two tyrosine units, is now well established.[3,4] In the present Paper the later stages of the biosynthetic pathway, leading from reticuline (I) to thebaine (VI), will be discussed. Evidence for the conversion of thebaine into codeine (IV; R = Me) and thence into morphine (IV; R = H) has been reviewed.[3]

Reticuline (I) has two phenolic hydroxyl groups in the positions required [8] for oxidative coupling to give the morphine skeleton, and it appeared, therefore, to be a likely intermediate in the biosynthetic pathway. (+)-Reticuline was isolated from *Anona reticulata*,[9] and the synthesis of the racemate has been well described.[10-13] Modifications used in the radiochemical syntheses are given in the Experimental section.

In preliminary experiments at Imperial College, (±)-reticuline, labelled with ^{14}C in the *N*-methyl group and with ^{3}H at C-1, was fed to flowering *Papaver somniferum* plants.[1] Incorporation (0·13%) into morphine was observed and was greater than that (0·08%) when 2-labelled (±)-tyrosine was fed under the same conditions. The ^{3}H : ^{14}C ratio in the isolated morphine was 83% of that in the reticuline. We at first considered that neither extensive dehydrogenation nor fragmentation of the precursor had occurred (see further below). Zeisel demethylation of the morphine established that all the ^{14}C activity was in the *N*-methyl group. Independent studies,[2,14] carried out during the same season in

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[1] D. H. R. Barton, G. W. Kirby, W. Steglich, and G. M. Thomas, *Proc. Chem. Soc.*, 1963, 203.
[2] A. R. Battersby, R. Binks, D. M. Foulkes, R. J. Francis, D. J. McCaldin, and H. Ramuz, *Proc. Chem. Soc.*, 1963, 203.
[3] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189.
[4] D. H. R. Barton, Hugo Muller Lecture, *Proc. Chem. Soc.*, 1963, 293.
[5] A. R. Battersby, Donegani Lectures on Biosynthesis, Milan, Sept. 1962; Accad. Naz. dei Lincei, VII° Corso Estivo di Chimica, 1964, 37.
[6] D. H. R. Barton and G. W. Kirby, Donegani Lectures on Biosynthesis, Milan, Sept. 1962; Accad. Naz. dei Lincei, VII° Corso Estivo di Chimica, 1964, 17.
[7] D. H. R. Barton, *Pure and Appl. Chem.*, 1964, **9**, 35.
[8] D. H. R. Barton and T. Cohen, "Festschrift A. Stoll," Birkhauser, Basel, 1957, p. 117.
[9] K. W. Gopinath, T. R. Govindachari, B. R. Pai, and N. Viswanathan, *Chem. Ber.*, 1959, **92**, 776.
[10] M. Tomita and I. Kikkawa, *Pharm. Bull. Japan*, 1956, **4**, 230; *J. Pharm. Soc. Japan*, 1957, **77**, 195.
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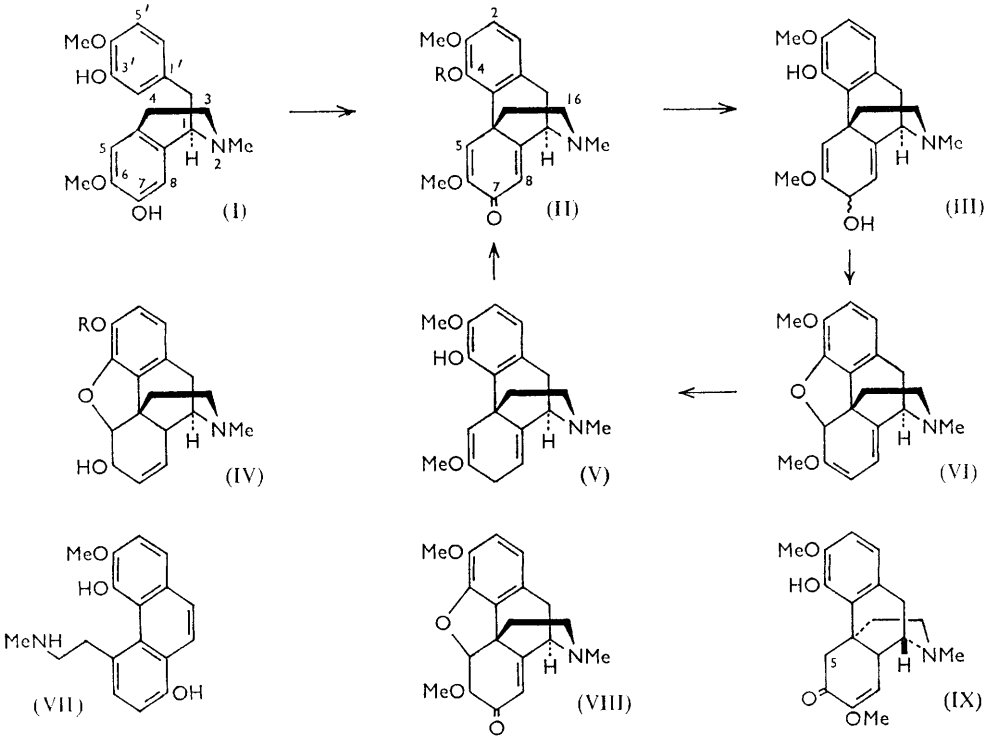
[12] J. Kunitomo, *J. Pharm. Soc. Japan*, 1961, **81**, 1253.
[13] M. K. Jain, *J.*, 1962, 2203.
[14] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J.*, 1964, 3600.

Liverpool, employed (±)-[3-^{14}C]reticuline. Efficient conversion into morphine was observed, the incorporation (7·3%) being greater than that for *N*-nor-reticuline (3·2%) and for norlaudanosoline (tris-nor-reticuline) (2·2%). The morphine was degraded (cf. ref. 14 and earlier Paper cited therein) by way of α-codeimethine to the phenanthrene derivative, methylmorphenol, which was essentially inactive. All the radioactivity was therefore in the 15,16-bridge and was presumably confined, as expected, to C-16. These results present powerful support for the idea that reticuline is the immediate biosynthetic



precursor of the morphine skeleton. However, it could be argued that de-*O*-methylation had taken place in the plant to give a simpler benzylisoquinoline which was then converted into morphine by some other route. To test this point, quintuply labelled (±)-reticuline was synthesised, at Imperial College, with ^{14}C in both methoxyl groups, in the *N*-methyl group, and at C-3, and with ^{3}H at C-1. A short-term feeding experiment using *Papaver somniferum* Noordster gave radioactive thebaine (VI) (0·09% incorporation based on ^{14}C) which was counted for ^{14}C and ^{3}H. The ^{14}C : ^{3}H ratio was similar to that of the precursor (Table 1).* Selective Zeisel determination of the *O*- and *N*-methyl groups gave methoxyl and *N*-methyl activities which showed that little or no de-*O*-methylation or de-*N*-methylation had occurred during biosynthesis. The individual activities of the two methoxyl groups in thebaine were obtained by acid-catalysed rearrangement of the alkaloid to thebenine (VII) followed by a second selective Zeisel determination. The activity at C-3 was obtained by difference, and served to exclude the remote possibility that partial and

* More recent experience with the counting equipment used in this work has shown that ^{3}H : ^{14}C ratios of less than 3 : 1 cannot be determined with great precision. The morphine from the preliminary, double labelling, experiment certainly retained a large amount of tritium but the measured value (83%) must be treated with reserve. We now consider that the amount of tritium present in the quintuply labelled reticuline (see later) was too small to give a reliable indication of tritium retention in the derived thebaine. The fate of hydrogen at C-1 in benzylisoquinoline precursors is presently being studied in greater detail by the Liverpool group.

TABLE 1

Labelling pattern in reticuline and thebaine
(values in parentheses were obtained by difference)

Compound	NMe	4′-OMe	6-OMe	C-3	^3H *
Reticuline	1·00	0·86	0·93	1·29	2·18
		1·69			
Thebaine	1·00			(1·40)	2·26
Thebenine	1·00	0·99	(0·82) †	(1·56)	—

* See footnote on p. 2424. † Value for 6-OMe in thebaine obtained by direct comparison of the methoxyl values of thebaine and thebenine disregarding the ratio of *N*-methyl activity to total radioactivity in the two compounds.

equal loss of the other four labels had occurred. Clearly, reticuline is incorporated into thebaine without degradation.

According to the original proposal,[8] reticuline should be oxidised initially to the dienone (II; R = H). By analogy with the properties of Pummerer's ketone [15] and narwedine,[16] this would be expected to cyclise spontaneously to yield the enone (VIII) which, after reduction and dehydration, would give thebaine. At the time this work began the dienone (II; R = H), or its tautomer (VIII), were not known compounds. A number of routes were explored for their partial synthesis from accessible morphine alkaloids.

After a successful synthesis of the dienone (II; R = H) had been devised, by the Imperial College group,[1] Professor R. A. Barnes (University of Brazil, personal communication) reported the isolation of a dienone alkaloid, salutaridine, from *Croton salutaris*. A direct comparison of the natural alkaloid with our material showed them to be identical; the name salutaridine will be used in the sequel. The synthesis of salutaridine was achieved as follows. Reduction of thebaine (VI) with sodium in liquid ammonia [17,18] gave dihydrothebaine-ϕ (V). This phenol was acetylated and oxidised successively with selenium dioxide and manganese dioxide to give *O*-acetylsalutaridine (II; R = Ac). Mild alkaline hydrolysis then gave the highly crystalline salutaridine having physical properties (see below) consistent with the phenolic structure (II; R = H). Ring-closure to give the enone (VIII) did not take place in either acidic or basic solution, and it is clear that the open form is thermodynamically the more stable. This observation suggested that closure of the benzofuran ring would require a separate biochemical step, and a modification of the original biosynthetic pathway became necessary. In fact, an alternative route had already been suggested [3,19] before the synthesis of salutaridine by the Imperial College group had been completed. Chemical support for the modified route was soon obtained. Reduction of salutaridine with sodium borohydride gave a readily separable mixture of epimeric alcohols, salutaridinols-I and -II (III) in almost equal amounts. The highly crystalline salutaridinol-I, m. p. 227—229° (decomp.), crystallised readily from the mixture upon addition of ethyl acetate. Both epimers could be separated by chromatography on alumina, salutaridinol-I being eluted first. Salutaridinol-II, m. p. 132—140° (after sublimation), proved difficult to purify, and specimens deteriorated when exposed to air. The infrared and n.m.r. spectra (see Tables 2 and 3) of the alcohols were closely similar and were consistent only with the structures (III). Their relative stereochemistry is at present being investigated at Imperial College. Both alcohols were readily dehydrated in aqueous acidic solution to give thebaine in 30—40% yield. Cyclisation proceeded at a measurable rate even at pH 5 and was faster for salutaridinol-I both at this pH and in hydrochloric acid solution (see Experimental section).

Once supplies of salutaridine and the derived dienols were available it became possible

[15] D. H. R. Barton, A. M. Deflorin, and O. E. Edwards, *J.*, 1956, 530.
[16] D. H. R. Barton and G. W. Kirby, *J.*, 1962, 806.
[17] K. W. Bentley and R. Robinson, *Experientia*, 1950, **6**, 353; K. W. Bentley, R. Robinson, and A. E. Wain, *J.*, 1952, 958.
[18] G. Stork, *J. Amer. Chem. Soc.*, 1951, **73**, 504; 1952, **74**, 768.
[19] D. Ginsburg, "The Opium Alkaloids," Interscience, New York, 1962, p. 91.

TABLE 2

Nuclear magnetic resonance spectra in deuterochloroform (d = doublet; t = triplet)
(for further details see Experimental section)

Compound	NCH_3	OCH_3	OCH_3	1 or 2		5	7	8
Dihydrothebaine-ϕ (V)	7·61	6·36	6·16	3·39	3·37	3·87	—	4·38 (t, 3·5 c./sec.)
Salutaridinol-II (III)	7·55	6·30	6·12	3·32	3·30	3·60	5·41(d)	4·19(d)
							3·2 c./sec.	
Salutaridine (II; R = H)	7·55	6·26	6·12	3·32	3·28	2·44	—	3·68
O-Acetylsalutaridine (II; R = Ac)	7·54 (Also MeCO·O at 7·60)	6·23	6·12	3·11		3·04	—	3·69
Sinomenine (IX)	7·54	6·53	6·21	3·42	3·40	5·66(d) 7·55(d)	15·5 c./sec.	4·53 (d, 2 c./sec.)

TABLE 3

Nuclear magnetic resonance spectra in dimethylformamide (d = doublet; t = triplet)
(for further details see Experimental section)

Compound	OCH_3	OCH_3	1 or 2		5	7	8
Dihydrothebaine-ϕ (V)	6·42	6·20	3·48(d)	3·21(d)	3·73	—	4·47 (t, 3·5 c./sec.)
			8·3 c./sec.				
O-Acetyldihydrothebaine-ϕ [*O*-acetate of (V)]	6·37	6·23	3·02		4·47	—	4·42 (t, 4·0 c./sec.)
Salutaridinol-I (III)	6·41	6·20	3·44(d)	3·17(d)	3·58	5·52(d)	4·35(d)
			8·5 c./sec.			4·2 c./sec.	
Salutaridinol-II (III)	6·38	6·18	3·45(d)	3·17(d)	3·52	5·59(d)	4·35(d)
			8·3 c./sec.			3·3 c./sec.	
Salutaridine	6·33	6·19	3·36(d)	3·09(d)	2·27	—	3·77
			8·2 c./sec.				
1-Bromosalutaridine	6·34	6·13	—	2·78	2·32	—	3·73

to make a critical experimental test of the postulated biosynthetic pathway, (II) ⟶ (III) ⟶ (VI). It was decided that independent and parallel experiments should be made by our two groups. Agreement between the two sets of results would then provide overwhelming evidence for, or against, the biogenetic proposals, and would also be most valuable in deciding which, if either, of the salutaridinols is the biological precursor of thebaine. For reasons of latitude, rather than light-intensity, poppies bloom earlier in London than in the Wirral Peninsula. The results of the Imperial College group will therefore be discussed first.

Dihydrothebaine-ϕ (V) was treated with hot alkaline tritiated water,[20] to give the [1-^{3}H]derivative. A parallel experiment using D_2O showed (n.m.r. spectrum) that the isotope was exchanging only with the hydrogen at C-1. Oxidation of the *O*-acetate in the usual way gave the desired [1-^{3}H]salutaridine. Bromination of this material yielded 1-bromosalutaridine containing, as expected, negligible amounts of tritium. [1-^{3}H]Salutaridinols-I and -II were obtained by reduction of the tritiated ketone with sodium borohydride, and [7-^{3}H]salutaridinols-I and -II by reduction of radiochemically inactive salutaridine with sodium [^{3}H]borohydride. In the first feeding experiment, [1-^{3}H]-salutaridine was injected into the capsules of *Papaver somniferum* Noordster. The plants were worked up after 4 days, and the major morphine alkaloids isolated. Efficient incorporation into thebaine was observed. Activity was also found in the codeine and morphine (Table 4). It was also shown, by dilution with inactive material, that the basic extract contained 40% of unmetabolised [1-^{3}H]salutaridine. Allowing for the inevitable losses during extraction, we can say that about 10% of the metabolised precursor was converted into the morphine alkaloids in this experiment. This incorporation is very high compared with those usually experienced in tracer studies with the higher plants.

[20] Cf. G. W. Kirby and L. Ogunkoya, unpublished observations; see also A. Murray and D. L. Williams, " Organic Syntheses with Isotopes," Part II, Interscience, New York, 1958, p. 1652.

TABLE 4

Feeding experiments with salutaridine derivatives

Alkaloid	Incorporations (%)		
	Salutaridine *	Salutaridinol-I (control)	Salutaridinol-II (control)
Thebaine	4·5	7·5(1·34)	0·28(1·81)
Codeine	0·70	—	—
Morphine	0·43	—	—

* Salutaridine was recovered in 40% yield from this experiment.

The biosynthetic thebaine was diluted with inactive alkaloid and converted, by our usual sequence, into salutaridine which contained 97% of the original activity. Bromination then gave essentially inactive 1-bromosalutaridine; thus, biosynthesis had proceeded without " scrambling " of the radio-label. The next step of the biosynthetic sequence was tested in the following way. Samples of [1,7-^{3}H]salutaridinols-I and -II, containing almost equal amounts of activity at positions 1 and 7, were prepared by mixing appropriate quantities of the singly labelled species. These precursors were fed separately to poppies in aqueous solution at *ca.* pH 6, a small portion of each solution being set aside and kept as a control at room temperature during the experiment. High incorporation of salutaridinol-I into thebaine was observed, the conversion being 5·6 times greater than that occurring in the control solution (Table 4). In contrast, salutaridinol-II was less efficiently converted in the plant than *in vitro*. Degradation of the thebaine, obtained from the salutaridinol-I feeding, by way of salutaridine to 1-bromosalutaridine, showed that 47% of the activity was at C-1 and the remainder at C-7. The precursor had 50·(5)% of its activity at C-1. Thus, the alcohol was incorporated intact into thebaine without preliminary oxidation to salutaridine. The very different incorporations observed with alcohols-I and -II strongly suggest that the cyclisation is enzymatically controlled, possibly through a phosphorylated intermediate,[2] and that salutaridinol-I is the biological presursor of thebaine.

The Liverpool group's approach began with biosynthetic [16-^{14}C]thebaine obtained from poppies fed with (±)-[3-^{14}C]norlaudanosoline (tris-nor-reticuline). This radioactive thebaine had been shown earlier to be labelled exclusively in the 16-position.[14] The biosynthetic alkaloid was converted, in the usual way, into [16-^{14}C]salutaridine and thence into [16-^{14}C]salutaridinols-I and -II. Specimens of the [7-^{3}H]salutaridinols were prepared as described above and mixed with the respective [16-^{14}C]dienols to give [7-^{3}H; 16-^{14}C]-salutaridinols-I and -II. The results of the feeding experiments are summarised in Table 5.

TABLE 5

Radioactive tracer experiments on *Papaver somniferum* plants

No. of plants; Year	3; 1963			3; 1963			3; 1963	
Amount fed (precursor)	0·255 μc ^{14}C; 3·75 μc ^{3}H (salutaridinol-I)			0·341 μc ^{14}C; 3·94 μc ^{3}H (salutaridinol-II)			0·215 μc ^{14}C (salutaridine)	
^{3}H : ^{14}C ratio	14·7 : 1			11·6 : 1			—	
	% Incorpn.	^{3}H : ^{14}C ratio	% Loss of ^{3}H	% Incorpn.	^{3}H : ^{14}C ratio	% Loss of ^{3}H	% Incorpn.	Incorpn. of salutaridinol-I : salutaridinol-II
Thebaine	1·74	14·9 : 1	1·36	0·12	11·3 : 1	2·6	3·90	14·5 : 1
Codeine	2·62	12·4 : 1	15·6	0·15	10·0 : 1	13·8	7·02	17·5 : 1
Morphine	2·43	12·0 : 1	18·4	0·13	10·0 : 1	13·8	3·72	18·7 : 1
Salutaridine	—	—	—	—	—	—	3·61	—

Very efficient (*ca.* 15%) conversion of salutaridine into the morphine alkaloids was observed, and again salutaridinol-I proved a much better precursor for thebaine than did its epimer. Careful measurement of the ^{3}H : ^{14}C ratios in the alkaloids derived from the salutaridinols revealed an interesting effect. The ratio in thebaine was the same, within experimental error, as that in the precursor. However, significant loss of tritium occurred during the conversion of thebaine into codeine. Further investigation of this effect may throw light

on intermediates involved in the transformation, neopinone and codeinone being, at present, likely possibilities.

Although salutaridine has never been detected in opium we have now been able to demonstrate the presence, in small amounts, in the flowering poppy. Part of the phenolic alkaloids from the batch of plants fed with [3-^{14}C]norlaudanosoline (see above) was diluted with radiochemically inactive salutaridine. After re-isolation and rigorous purification the salutaridine had an activity corresponding to a 0·034% incorporation of norlaudanosoline. Moreover, the alkaloid retained its activity after conversion into thebaine by way of the salutaridinols. A similar result was obtained at Imperial College from plants fed with (±)-[2-^{14}C]tyrosine. In this experiment, the incorporation into salutaridine was smaller (0·005%), but tyrosine is known [14] to be less efficient than norlaudanosoline as a precursor for the morphine alkaloids. Clearly, salutaridine is present in the plant but its synthesis and conversion into thebaine proceed at similar rates and the stationary concentration is therefore small.

We consider that the results reported in the present Paper, when combined with earlier work, define with considerable precision many of the important terminal steps in the biosynthesis of the opium alkaloids.

Nuclear magnetic resonance data for many of the compounds discussed in this Paper have been collected in Tables 2 and 3. Comment on these data and their interpretation is presented in the Experimental section.

Experimental

(*a*) Imperial College Group

Melting points were taken on a Kofler hot-stage apparatus. Unless otherwise specified, optical rotations were measured in chloroform, ultraviolet spectra in ethanol, and infrared spectra in chloroform. Chromatography was carried out on neutral Grade III alumina.

Counting Methods.—Compounds labelled with ^{14}C were counted as thin films by the method reported earlier.[21] Tritium activities were measured with a Tritium Scintillation Counter (Isotope Developments Ltd., Type 6012 A), samples being dissolved in dimethylformamide (0·1 ml.) and liquid scintillator (Nuclear Enterprises Ltd., type N.E. 213) (1·2 ml.). The counting procedure was calibrated using standard [1,2-$^{3}H_2$]-n-hexadecane, efficiences of *ca.* 20% being customary. Samples containing both ^{3}H and ^{14}C were counted by the scintillation method at two different voltage settings, one being optimal for ^{3}H and the other for ^{14}C. It was not possible to discriminate completely between the two isotopes in this way, and some additional error was introduced which became appreciable for ^{3}H : ^{14}C ratios of less than 3.

Radioactive Precursors and Alkaloids.—The methods and precautions described earlier [21] for radiochemical syntheses and degradations were adhered to in the present investigation. Multiply labelled precursors were generally prepared by mixing known amounts of the singly labelled species.

(±)-*Reticuline.*—The synthesis followed that worked out previously in Glasgow [13] with some modifications in the various radiochemical preparations. 4-Benzyloxy-3-methoxyphenyl-[1-^{14}C]acetonitrile was obtained from the corresponding substituted benzyl chloride with sodium [^{14}C]cyanide in dimethyl sulphoxide. Reduction of this nitrile with lithium aluminium hydride gave the required 4-benzyloxy-3-methoxyphen[1-^{14}C]ethylamine. The overall procedure did not differ in essential detail from that reported by Battersby *et al.*[14] 3-Benzyloxy-4-hydroxybenzaldehyde [21] and 4-benzyloxy-3-hydroxybenzaldehyde [21] were each methylated with [^{14}C]methyl iodide,[21] to give, respectively, *O*-benzylisovanillin and *O*-benzylvanillin labelled in the methoxyl groups. The labelled *O*-benzylvanillin was converted, through the derived nitrostyrene,[13] into 4-benzyloxy-3-[*methyl*-^{14}C]methoxyphenethylamine required for the usual [13] reticuline synthesis. The corresponding labelled *O*-benzylisovanillin was oxidised with potassium permanganate to give *O*-benzyl-[*methyl*-^{14}C]isovanillic acid [22] which was converted [11] into 3-benzyloxy-4-[*methyl*-^{14}C]methoxy-ω-diazoacetophenone. This diazo-ketone was used in the following two variants of the Wolff rearrangement.

[21] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J.*, 1963, 4545.
[22] A. Lovecy, R. Robinson, and S. Sugasawa, *J.*, 1930, 817.

*3-Benzyloxy-*N*-(4-benzyloxy-3-methoxyphenethyl)-4-methoxyphenylacetamide.*—(*a*) Freshly prepared silver oxide (10 mg.) was added to the diazo-ketone (24 mg.) and 4-benzyloxy-3-methoxyphenethylamine (from 25 mg. of the oxalate) in benzene (45 ml.). The mixture was heated with stirring at 60° for 3 hr. then at 80° for 30 min. Filtration and evaporation gave a crude product which was treated with charcoal in ethanol. Removal of the charcoal and evaporation gave essentially pure (infrared spectrum) amide (32 mg.), m. p. 135—136°.

(*b*) (with D. S. Bhakuni and G. E. Palmer). The diazo-ketone (148 mg.) and the phenethylamine (180 mg.) in dry benzene (30 ml.) were photolysed in a Pyrex flask, using a high-pressure mercury vapour lamp, for 3·5 hr. (infrared control). The benzene solution was washed with N-hydrochloric acid and with water, dried ($MgSO_4$), and evaporated. The residue crystallised from ethanol to give the desired amide as needles (188 mg.), m. p. and mixed m. p. 139—141°. More consistent results were obtained by this method than with procedure (*a*), and photolyses were performed successfully on a large (*ca.* 6 g. of diazo-ketone) scale.

(±)-[N-methyl-^{14}C; 1-^{3}H]-OO-*Dibenzylreticuline.*—1-(3-Benzyloxy-4-methoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline [obtained from its hydrochloride [12] (70 mg.) with aqueous sodium hydrogen carbonate and ether extraction] in benzene (5 ml.) was frozen, and [^{14}C]methyl iodide (*ca.* 3 mg.) distilled (vacuum line) in. The reaction mixture was sealed *in vacuo* and stirred at room temperature for 5 days during which time the desired methiodide separated. The methylation was completed by the addition of a large excess of inactive methyl iodide and a further 8 hr. stirring.

The precipitated [N-*methyl*-^{14}C]methiodide (49 mg.), m. p. 197—198° (lit.,[12] 196—198°), was collected and combined with a further amount (5 mg.) obtained from the mother-liquors. The combined product was reduced at 0°, in methanol (3 ml.), with sodium [^{3}H]borohydride (50 mg.) (see below for its preparation), left at room temperature for 1 hr., and then evaporated. Water (2 ml.) and 4N-sodium hydroxide (0·2 ml.) were added, and the suspension extracted with ether (5 × 2 ml.). Evaporation gave the required *OO*-dibenzylreticuline (32 mg.) which was purified by chromatography on alumina. Elution with benzene gave pure material (29 mg.) which crystallised under light petroleum (b. p. 40—60°) as needles, m. p. 86—88° (lit.,[13] 89°).

(±)-*Reticuline.*—(±)-*OO*-Dibenzylreticuline (1·0 g.), in ethanol (50 ml.) containing concentrated hydrochloric acid (1 ml.), was hydrogenated at room temperature over 10% palladised charcoal (1·0 g.) for 30 min. (2 mol. uptake). The resulting solution was filtered and evaporated, and the residue treated with excess of N-sodium hydrogen carbonate and extracted with chloroform (3 × 20 ml.). The extract was dried (Na_2SO_4) and evaporated, to give (±)-reticuline as a colourless glass (634 mg.) having an infrared spectrum identical with that of material obtained [12] by debenzylation with hydrochloric acid. (±)-Reticuline formed a picrate which separated slowly from ethanol as prisms, m. p. 188—190° (lit.,[13] 190—192°) (Found: C, 54·0; H, 5·0; N, 10·3. Calc. for $C_{25}H_{26}N_4O_{11}$: C, 53·75; H, 4·7; N, 10·0%).

Hydrogenolysis proceeded satisfactorily on the small scale used in radiochemical preparations. Purification of small quantities (*ca.* 10 mg.) was effected by chromatography on alumina, (±)-reticuline being eluted with chloroform–ethanol (95 : 5). Elution was followed both by ultraviolet absorption at 280 mμ and by scintillation counting. The coincidence of absorption and radioactivity elution curves was taken as proof of radiochemical purity.

O-*Acetyldihydrothebaine-*ϕ.—Dihydrothebaine-ϕ [17] (16·4 g.) in pyridine (50 ml.) and acetic anhydride (10 ml.) was left for 2 hr. at 0° and then at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue shaken with N-sodium hydrogen carbonate and extracted with chloroform. The extracts were dried (Na_2SO_4) and evaporated, to give a brown syrup which was chromatographed on alumina (500 g.). Elution with benzene–ethyl acetate (4 : 1) gave the desired *O*-acetate as an oil (15·4 g.) which did not crystallise, it showed characteristic infrared bands at 1762 (ester carbonyl) and at 1703 and 1663 (1,4-diene [18]) cm.$^{-1}$.

Salutaridine (*7-Oxodihydrothebaine-*ϕ).—Freshly sublimed selenium dioxide (5·3 g.) in ethanol (15 ml.), and *O*-acetyldihydrothebaine-ϕ (15·4 g.) in dioxan (300 ml.), were heated under reflux for 8 hr. and then left at room temperature overnight. Selenium (2·88 g.) was filtered off, and the solvent evaporated *in vacuo*. The residue was treated with charcoal (5 g.) in refluxing benzene (200 ml.), and the resulting clarified solution filtered and evaporated. This residue, in chloroform (200 ml.), was shaken with manganese dioxide (B.D.H.; 60 g.) for 30 min. at room temperature and then filtered through Celite and evaporated. The remaining dark brown material (12·5 g.) was heated under reflux in ethanol (120 ml.) containing yellow ammonium

sulphide solution (4 ml.) for 1 hr. After filtration through Celite the cooled solution was diluted with ethanol (80 ml.) and treated with 2N-sodium hydroxide (60 ml.) at room temperature overnight. The ethanol was evaporated, and the residue in water (200 ml.) washed with ether. The phenolic dienone, precipitated by addition of excess solid carbon dioxide, was then extracted into chloroform (2 l.). The extract was dried ($MgSO_4$), evaporated, and the residue (9·7 g.) chromatographed over alumina. Elution with benzene–ethyl acetate (1 : 1) gave *salutaridine*. This crystallised from ethyl acetate as rods (5·7 g.), m. p. 197—198°, $[\alpha]_D$ +111° (*c* 1·69 in ethanol), +104° (*c* 1·50 in acetic acid) (Found: C, 69·7; H, 6·4; N, 4·2. $C_{19}H_{21}NO_4$ requires C, 69·7; H, 6·5; N, 4·3%). Salutaridine gave a deep blue colour with ethanolic ferric chloride and had an infrared spectrum (strong bands at 3560, 1671, 1644, and 1624 $cm.^{-1}$) and ultraviolet spectrum ($\lambda_{max.}$ 240 and 277 mμ; ε, 17,800 and 5700, respectively) consistent with the phenolic dienone formulation. The n.m.r. spectrum (see below) provided conclusive proof of this structure. The physical properties of our material were identical with those of the natural alkaloid (Professor R. A. Barnes, personal communication). Acetylation with acetic anhydride in pyridine in the usual way gave O-*acetylsalutaridine*, m. p. 171°, $[\alpha]_D$ +120° (*c* 1·26 in ethanol) (Found: C, 68·3; H, 6·3; N, 3·8. $C_{21}H_{23}NO_5$ requires C, 68·3; H, 6·3; N, 3·8%).

1-*Bromosalutaridine*.—Bromine (64 mg.) in chloroform (5 ml.) was added dropwise with stirring to salutaridine (120 mg.) in chloroform (30 ml.). After 4 hr. at room temperature the solution was washed with N-sodium hydrogen carbonate and with water and then evaporated. The residue was filtered through alumina in chloroform–ethyl acetate (1 : 1). The eluted 1-*bromosalutaridine* crystallised from chloroform as a solvate, sintering at 120—130°, resolidifying and remelting at 196—197°, $\nu_{max.}$ 3550, 1671, 1646, and 1625 $cm.^{-1}$ (Found: C, 45·3; H, 4·0; N, 3·4. $C_{19}H_{20}BrNO_4,CHCl_3$ requires C, 45·7; H, 4·0; N, 2·7%). The position of bromination was established by preparation of 1-bromosalutaridine from the dienone labelled specifically at C-1 with deuterium or tritium (loss of isotope; see below).

Salutaridinols-I and -II.—Salutaridine (900 mg.), in methanol (60 ml.), was treated with sodium borohydride (2·5 g.) at 0° with stirring. After 2 hr. at 0° and 2 hr. at room temperature the methanol was evaporated and the residue was shaken with water and chloroform. Evaporation of the chloroform extract gave a crystalline mixture of alcohols (980 mg.). A thin-layer chromatogram on alumina G (Merck), developed with chloroform, showed two components, R_F 0·25 (salutaridinol-I) and 0·11 (salutaridinol-II), giving orange spots with the Dragendorff reagent,[23] and no unreacted dienone. The mixture was digested with ethyl acetate (80 ml.) and left at room temperature overnight. The crystals of *salutaridinol-I* (405 mg.) which separated were filtered off and recrystallised from methanol, to give needles, m. p. 223—225° (decomp.). After sublimation (170°/10^{-4} mm.) the alcohol-I had m. p. 227—229° (decomp.), $[\alpha]_D$ +42·5° [*c* 1·8 in acetic acid (no change of rotation in 10 min.)], $\nu_{max.}$ 3620, 3560, and 1703 and 1661 (1,4-dienol [18]), 1619 and 1588 $cm.^{-1}$ (Found: C, 69·0; H, 7·1; N, 4·5. $C_{19}H_{23}NO_4$ requires C, 69·3; H, 7·0; N, 4·25%).

The ethyl acetate filtrate (above) was evaporated and the residue chromatographed over alumina (180 g.). Elution with chloroform–ethyl acetate (1 : 1) gave a fraction (47 mg.) which crystallised from benzene–light petroleum as long needles, m. p. >320° (darkening at 240°). The infrared spectrum was closely similar to that of salutaridinol-I but showed additional strong bands at 2300 and 2400 $cm.^{-1}$. The compound did not react with Dragendorff's reagent and appeared to be a 1 : 1 complex of salutaridinol-(I or II) with borane (Found: C, 66·0; H, 7·5; N, 3·9. Calc. for $C_{19}H_{26}BNO_4$: C, 66·5; H, 7·6; N, 4·1%). It was not investigated further. Elution with chloroform gave salutaridinol-I (20 mg.), while chloroform–methanol (98 : 2) eluted *salutaridinol-II* (391 mg.) as a syrup which crystallised slowly under ether. Rechromatography gave a resin, $[\alpha]_D$ +24° (*c* 1·51 in acetic acid), which showed one component on a thin-layer chromatogram and had an infrared spectrum almost indistinguishable from that of salutaridinol-I. This material proved difficult to crystallise, readily formed solvates, and darkened on prolonged exposure to air. Specimens of pure alcohol-II which had been kept in air were found to contain traces of salutaridine. After careful rechromatography salutaridinol-II sublimed (150—170°/10^{-4} mm.) to give material, m. p. 132—140° (Found: C, 70·0; H, 7·1; N, 4·2. Calc. for $C_{19}H_{23}NO_4$: C, 69·3; H, 7·0; N, 4·3%). The carbon value diminished rapidly when the analytical specimen was exposed to air (C, 68·2% after *ca.* 24 hr. and 66·8% after *ca.* 36 hr.). The n.m.r. spectrum of salutaridinol-II was very similar to that of its epimer and left no doubt as to its structure.

[23] Z. Margasinski, A. Szymanska, and I. Wasilewska, *Acta polon. Pharm.*, 1955, **12**, 65.

Acid-catalysed Cyclisation of the Salutaridinols.—Salutaridinol-I (50 mg.) was treated with N-hydrochloric acid (4 ml.) at room temperature for 30 min. The solution was made strongly alkaline with sodium hydroxide and extracted with chloroform. Chromatography of the evaporated extract on alumina (50 g.) eluting with benzene–ethyl acetate (1 : 1) gave thebaine (17 mg., 37%) (m. p. and mixed m. p., ultraviolet and infrared spectra). Thebaine itself was not significantly affected by N-hydrochloric acid under these conditions. Salutaridinol-II, when treated in the same way, also gave thebaine (31%). Salutaridinol-I was unchanged in water at 100° during 8 hr.

Each of the epimeric alcohols (22 mg.) was solvolysed, in 50% aqueous acetic acid (1 ml.) made either 0·1- or 0·2-N with respect to hydrochloric acid, at 26°. The reaction was followed by optical rotation, good apparent first-order plots being obtained over 2 half-lives. All four reaction mixtures approached the same final optical rotation ($[\alpha]_D$ *ca.* −36°). In 0·1-N-acid the half-lives for alcohols-I and -II were 34 and 68 min., respectively. In 0·2-N the corresponding values were 16·5 and 26 min.

[1-^{3}H]*Salutaridine.*—Conditions for the isotopic exchange of the hydrogen *para* to the phenolic hydroxyl group in dihydrothebaine-ϕ were investigated using alkaline deuterium oxide solutions. The complete exchange of *one* aromatic hydrogen under the following conditions was verified by n.m.r. spectroscopy. Dihydrothebaine-ϕ (500 mg.), potassium t-butoxide (90 mg.), dimethylformamide (2·5 ml.), and [^{3}H]water (0·5 ml., 0·25c) were sealed under nitrogen in a Carius tube and heated at 100° for 5 days. The solvent was removed under reduced pressure and the residue dissolved in water. The phenol was liberated by addition of excess solid carbon dioxide, extracted with chloroform, and acetylated with acetic anhydride (0·3 ml.) in pyridine (2 ml.) in the usual way. After chromatography (as above) *O*-acetyl-[1-^{3}H]dihydrothebaine-ϕ (385 mg.) was obtained as a glass (3·6 mc/mmole). Oxidation of this material with selenium dioxide and manganese dioxide (as above) gave pure [1-^{3}H]salutaridine (148 mg.; 4·4 mc/mmole). Bromination of [1-^{3}N]salutaridine in chloroform gave 1-bromosalutaridine having <1% of the original activity.

[1-^{3}H]*Salutaridinols-I and -II.*—[1-^{3}H]Salutaridine (30 mg.) was reduced in ethanol with sodium borohydride (40 mg.) at 0°. After 2 hr. at 0° and 3 hr. at room temperature the mixture of dienols was isolated in the usual way and separated by chromatography. [1-^{3}H]Salutaridinol-I (13 mg.; 4·0 mc/mmole) and [1-^{3}H]salutaridinol-II (13·5 mg.; 3·9 mc/mmole) were obtained.

Sodium [^{3}H]*Borohydride.*[24]—Trimethylamine borine [25] (150 mg.) in ether (1·5 ml.) was shaken vigorously with [^{3}H]water (0·5 ml.; 0·25c) and sulphuryl chloride (0·013 ml.) for 6 hr. at room temperature. The ether layer was separated, dried (Na_2SO_4), and evaporated. The residue was sublimed to give crystalline trimethylamine [^{3}H]borine (123 mg.; 5·2 mc/mmole, *i.e.*, 1·7 mc/mg. atom labelled hydrogen). This material was treated with sodium methoxide (from 39 mg. sodium) in diglyme (4 ml.), under dry nitrogen, at 145°, with stirring for 2·5 hr. The precipitated salts were separated by centrifugation and were washed with warm diglyme. The combined diglyme solutions were evaporated (0·1 mm.) to give a semicrystalline mass which was used directly in the folowing preparation.

[7-^{3}H]*Salutaridinols-I and -II.*—Salutaridine (30 mg.) was reduced with sodium [^{3}H]borohydride (as above) to give [7-^{3}H]salutaridinol-I (11 mg.; 1·2 mc/mmole) and [7-^{3}H]salutaridinol-II [10 mg.; 1·2(5) mc/mmole].

Nuclear Magnetic Resonance Spectra of Salutaridine Derivatives.—The spectra were run in deuterochloroform or dimethylformamide on a Varian A-60 instrument (on permanent loan from the Wellcome Trust) using tetramethylsilane (10 τ) as an internal standard. Line positions of methyl, aromatic, and olefinic protons are given in τ units, and multiplicities (doublet or triplet) are shown in parentheses (d or t) (Tables 2 and 3). Protons attached to C-5 are strongly deshielded by the nearby aromatic ring [26] and appear *ca.* 2 p.p.m. downfield from their normal position. This is strikingly illustrated by the methylene protons at C-5 in sinomenine; one (5·66 τ) must lie close to the plane of the aromatic ring and absorbs in a quite different region of the spectrum from the other (7·55 τ). Again, the olefinic proton (C-8) of sinomenine appears 2·09 p.p.m. upfield from the otherwise similar (C-5) proton in salutaridine. Acetylation of the phenolic hydroxyl group causes shielding (*ca.* 0·6 p.p.m.) of a C-5 olefinic proton (compare dihydrothebaine-ϕ, salutaridine, and their *O*-acetates). The aryl proton bands of all phenols in

[24] Cf. R. E. Davis, A. E. Brown, R. Hopmann, and C. L. Kibby, *J. Amer. Chem. Soc.*, 1963, **85**, 487.
[25] G. W. Schaeffer and E. R. Anderson, *J. Amer. Chem. Soc.*, 1949, **71**, 2143.
[26] Cf. S. Goodwin, J. N. Shoolery, and L. F. Johnson, *Proc. Chem. Soc.*, 1958, 306.

this series separate when the solvent is changed from chloroform to dimethylformamide. In chloroform the two aryl protons are barely resolved ($\Delta\tau \simeq 0{\cdot}02$) while in dimethylformamide a well defined AB quartet ($\Delta\tau \simeq 0{\cdot}27$, J_{AB} 8·3 c./sec.) is observed. This effect is not seen with *O*-acetyldihydrothebaine-ϕ and may arise from ionisation of the phenol in the relatively basic solvent, dimethylformamide. In all these compounds the band in the region 6·1—6·2 τ may be assigned to the aromatic methoxyl group.

Infrared Spectra of Salutaridine Derivatives.—Salutaridine shows a sharp strong band at 1494 cm.$^{-1}$. This band disappears and is replaced by another, equally strong but broader, at 1475 cm.$^{-1}$, when the alkaloid is deuterated at C-1. The same effect is noticed in 1-bromosalutaridine and in the dimer obtained by oxidation of salutaridine. Deuteration of dihydrothebaine-ϕ also produces a similar spectral change which appears therefore to be characteristic of substitution at C-1 in this series.

Feeding Experiments with (±)-*Reticuline.*—(*a*) [1-^{3}H; *N-methyl*-^{14}C]Reticuline (1962). Two flowering plants of *Papaver somniferum* (from commercial seeds of no stated variety) were fed with an aqueous solution, at pH 6, of the doubly labelled precursor (10 mg., 0·02 mc) by hypodermic injection into the seed capsules. After 7 days the whole plants (wet wt. 120 g.) were extracted for morphine following Leete's procedure.[27] The radioactive morphine (39 mg.) was converted into its picrate, m. p. 163—165°, which was recrystallised twice from ethanol. The specific activity dropped by 12% after the first recrystallisation then stayed constant (relative molar activity of ^{14}C, 1·0). The free base was heated in acetic anhydride (0·3 ml.) at 100° for 15 min. and the reaction mixture then evaporated *in vacuo*. The residue of diacetylmorphine crystallised from ethyl acetate as cubes, m. p. 172—173° (relative activity of ^{14}C, 1·02). *N*-Methyl determination,[21] on morphine, gave radioactive methyltriethylammonium iodide (relative activity, 0·94). The incorporation of (±)-reticuline into morphine, based on ^{14}C, was 0·13%. The ^{14}C : ^{3}H ratios on the precursor and on the derived diacetylmorphine were measured, side by side, by the scintillation method. The values were 2·0 : 1 and 2·6 : 1, representing an apparent 83% retention of tritium. In a parallel experiment (±)-[2-^{14}C]tyrosine was also incorporated (0·08%) into morphine.

(*b*) Multiply labelled reticuline (1963). A specimen of reticuline, labelled with ^{14}C at the *N*-methyl group, both methoxyl groups, and at C-3, and with ^{3}H at C-1, was prepared by mixing the appropriate singly labelled species with [1-^{3}H; *N-methyl*-^{14}C]reticuline. This precursor was fed to *Papaver somniferum* Noordster and, after 3 days, the thebaine isolated. The procedure used is described in detail for the salutaridine experiments (below). The incorporation into thebaine, based on the total ^{14}C activity, was 0·09%. The purified, diluted alkaloid (40 mg.) was counted for ^{14}C and ^{3}H and the combined methoxyl and the *N*-methyl activities determined by the usual Ziesel method. The thebaine was converted into thebenine [28] which was also counted and assayed for *N*- and *O*-methyl activity. The chain label activity (C-3 in reticuline, C-16 in thebaine) was obtained by difference and is consequently less accurately known. The labelling pattern of the precursor and the derived thebaine is summarised in Table 1. In the precursor the total *N*-methyl activity was 0·011 mc and this has been given the arbitrary value of 1·00. The *N*-methyl activity in thebenine was 93% of the corresponding value in thebaine. Again, both are assigned the value 1·00 to make comparison easier.

Feeding Experiments with Salutaridine Derivatives (1963).—All precursors were fed to flowering plants of *Papaver somniferum* Noordster by hypodermic injection of their aqueous solutions (pH 6) into the seed capsules. In preliminary experiments, it was found that only small amounts of activity were transported into the main body of the plant during the relatively short (3—4 days) period allowed for metabolism. For this reason only the excised capsules, together with a short (*ca.* 5 cm.) section of stem, were extracted for alkaloids. All isolated alkaloids were checked for purity by comparison with authentic specimens. Thin-layer chromatography, on alumina G (Merck), with chloroform as a developing solvent, provided a useful test of purity. The chromatograms were sprayed with the Dragendorff reagent. Typical R_F values (varying with the age of the plate) are given (salutaridine and codeine tailed somewhat): salutaridine 0·55; thebaine 0·65; codeine 0·30; morphine 0—0·1; papaverine 0·80.

[1-^{3}H]*Salutaridine.*—The precursor (5 mg.; 0·068 mc) was fed to 8 seed capsules of 4 plants.

[27] E. Leete, *J. Amer. Chem. Soc.*, 1959, **81**, 3948.
[28] O. Hesse, *Annalen*, 1870, **153**, 47.

After 4 days the capsules (wet wt. 48 g.) were frozen under liquid nitrogen, powdered, and soaked in 0·5N-hydrochloric acid (80 ml.) overnight at room temperature. Inactive salutaridine, thebaine, codeine, and morphine (40 mg. of each) were added to the extract which was then filtered through Celite. The filtrate was washed with ether and then made alkaline with sodium hydroxide (8 g.) and extracted thoroughly with ether (3 × 100 ml.). The extracts were dried (Na_2SO_4) and evaporated to give a residue (74 mg.) consisting mainly of thebaine and codeine. This material was chromatographed on alumina (60 g.). Elution with benzene–ethyl acetate (7 : 3) gave thebaine (40 mg.) which was further purified by chromatography, and counted (4·5% incorporation of the precursor). Elution with chloroform gave codeine (27 mg.). Rechromatography gave pure material (0·70% incorporation). The original aqueous alkaline solution was next extracted with chloroform to give impure salutaridine (43 mg.). The pure alkaloid (22 mg.) was eluted from alumina (50 g.) with chloroform–ethyl acetate (1 : 1). Its specific activity corresponded to a 40% recovery of injected [1-^{3}H]salutaridine. Finally, the alkaline solution was treated with excess of solid carbon dioxide and extracted with chloroform–ethanol (9 : 1). The extracts were evaporated and the residue (83 mg.) in chloroform–ethanol (9 : 1) run on to a short column of alumina. The column was washed with chloroform and the morphine then eluted with ethanol. The alkaloid was crystallised from ethanol–chloroform and counted (0·43% incorporation). Evaporation of the mother-liquors from this crystallisation gave material having essentially the same specific activity as the crystalline morphine.

[1,7-$^3H_2$]*Salutaridinol-I.*—A mixture of [1-^{3}H]salutaridinol-I (1·2 mg., 0·0144 mc) and [7-^{3}H]salutaridinol-I (3·9 mg., 0·0141 mc) was suspended in water (0·5 ml.) and dissolved by the addition of a micro-drop of acetic acid. The solution was then adjusted to pH 6—7 with potassium carbonate. A few drops of this solution were set aside as a control and the remainder fed to 3 capsules of 2 Noordster plants. After 3 days the capsules (wet wt. 16 g.) were frozen and powdered, as before, and left overnight at room temperature in methanol (25 ml.) and *N*-sodium hydrogen carbonate (25 ml.). The resulting mass was filtered through Celite and the insoluble residue carefully washed with ethanol. The extracts were evaporated to give a dark green gum which was shaken with 2N-sodium hydroxide (50 ml.). Extraction with chloroform gave a green mass, shown by thin-layer chromatography to contain thebaine, codeine, and some salutaridinol-I. This material was diluted with inactive thebaine (20 mg.) and chromatographed on a long column (56 cm. × 1 cm.) of alumina. Elution with benzene–ethyl acetate (7 : 3) gave crude thebaine which was rechromatographed in a similar way. The eluted thebaine (19 mg.) in 0·1N-hydrochloric acid was washed with ether. The thebaine was recovered by basification and extraction with chloroform. After further purification by chromatography the pure alkaloid (17 mg.) was counted (7·5% incorporation).

The control solution after 3 days at room temperature of [1,7-$^3H_2$]salutaridinol-I was also treated with sodium hydrogen carbonate. Inactive thebaine (10 mg.) and salutaridinol-I (10 mg.) were added and the mixture extracted with chloroform. The dienone and dienol were separated by chromatography in the usual way and both counted. The recovered thebaine had 1·34% of the total activity.

[1,7-$^3H_2$]*Salutaridinol-II.*—This experiment was performed alongside that with the epimeric alcohol (above). The same chemical and radiochemical amounts of precursor were used and again 2 plants were fed and extracted after 3 days. The wet weight of the capsules was 18 g. The incorporation of salutaridinol-II into thebaine was 0·28%. The conversion in the control experiment was 1·81%.

Degradation of Thebaine from Salutaridine.—Thebaine (35 mg.), obtained from the [1-^{3}H]-salutaridine feeding, was diluted with inactive alkaloid (665 mg.) and the mixture crystallised (relative molar activity, 1·00). This material was converted, in the usual way, into salutaridine (r.m.a., 0·97). Bromination in chloroform gave 1-bromosalutaridine having virtually no activity (r.m.a. $<0·01$).

Degradation of Thebaine from Salutaridinol-I.—Diluted thebaine (400 mg.; r.m.a., 1·00), from the [1,7-$^3H_2$]salutaridinol-I feeding, was converted in the usual way into acetyldihydrothebaine-ϕ. The crude acetate (r.m.a., 0·86) was oxidised to give pure salutaridine (r.m.a., 0·47). The injected [1,7-$^3H_2$]salutaridinol-I had 50·5% of its activity at C-1.

Detection of Salutaridine in Papaver Somniferum.—(±)-[2-^{14}C]Tyrosine (0·02 mc) was fed in the usual way to Noordster plants. After 3 days the whole plants were macerated with N-hydrochloric acid containing inactive salutaridine (35 mg.). The mixture was filtered through Celite and the filtrate washed with ether, made strongly alkaline with sodium hydroxide,

and extracted with chloroform. The extracts were evaporated and the residue chromatographed three times on alumina to give pure salutaridine. After recrystallisation, salutaridine had an activity corresponding to a 0·0050% conversion from tyrosine. Reduction and treatment with acid (as above) converted the salutaridine into thebaine of essentially the same specific activity.

(*b*) University of Liverpool Group

All solutions in organic solvents were dried ($MgSO_4$) and evaporated under reduced pressure at $<40°$. M. p.s were determined on a Kofler hot-stage apparatus. Neutral alumina (activity III) was used throughout for chromatography.

Radioactive Assay.—The activities of singly labelled compounds (^{14}C or ^{3}H) were determined by scintillation counting in solution at the optimum voltage settings for each isotope. Samples were dissolved in methanol (0·1 ml.) and the scintillator solution (Nuclear Enterprises Ltd., type NE 213 or type NE 220) (3·9 ml.) was added. Internal standardisation with [1-^{14}C]-n-hexadecane and [1,2-$^{3}H_2$]-n-hexadecane allowed the counting efficiency to be determined for each sample. Doubly labelled materials (^{3}H, ^{14}C) were counted at two different voltages which had been selected by prior standardisation as the best for this purpose; again, internal standardisation was used.

Purity of Precursors and Isolated Alkaloids.—The criteria of chemical and radiochemical purity used below and the method used to calculate incorporations of activity were described [29] in Part III.

N-*Methyl*-1-(3-*hydroxy*-4-*methoxybenzyl*)-6-*methoxy*-7-*hydroxy*-1,2,3,4-*tetrahydro*[3-^{14}C] *isoquinoline* (*Reticuline*).—1-(3-Benzyloxy-4-methoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydro[3-^{14}C]isoquinoline hydrochloride [14] (44·6 mg., 0·067 mc) in methanol (1 ml.) was frozen (liquid air) in a glass tube and methanolic potassium hydroxide (0·19N; 1·1 equiv.) was added and frozen on top of the first solid. Methyl iodide (1 ml.) was then run into the tube, and, after this too had frozen, the tube was evacuated and sealed. The reaction mixture was heated at 50° for 5 hr., evaporated to low volume, treated with water (3 ml.), and the solid recrystallised from chloroform–ethyl acetate to yield the [3-^{14}C]methiodide (37·5 mg.), m. p. 199—201° [Found (on inactive material): C, 62·3; H, 5·3. Calc. for $C_{33}H_{34}INO_4$: C, 62·4; H, 5·4%].

This methiodide (37 mg.) in methanol (4 ml.) was treated with sodium borohydride (20 mg.) and then warmed at 40° for 0·5 hr. After the solution had been acidified, it was freed from methanol by evaporation and worked up for base by extraction into ether as usual to afford a gum. This was characterised in trial experiments using radio-inactive materials, as the *picrolonate* of *N*-methyl-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydro[3-^{14}C]isoquinoline by treatment of the base in methanol with a slight excess of picrolonic acid. The salt crystallised from methanol and had m. p. 182—184° (decomp.) (Found: C, 66·5; H, 5·75. $C_{43}H_{43}N_5O_9$ requires C, 66·7; H, 5·6%).

All the radioactive base above was shaken with hydrogen and 10% palladised charcoal (36 mg.) at a room temperature and pressure in a micro-hydrogenator. Uptake (2·85 mol.) was complete in 3·5 hr. N-Hydrochloric acid (1·1 equiv.) was added to the solution, the catalyst filtered off, and the filtrate evaporated to dryness to yield [3-^{14}C]reticuline hydrochloride (0·049 mc).

The final product was further characterised in radio-inactive runs by conversion into the picrate [13] in methanol, m. p. 194—196° (lit.,[13] 192—194°) (Found: C, 53·45; H, 4·8; N, 10·1. Calc. for $C_{35}H_{26}N_4O_{11}$: C, 53·7; H, 4·7; N, 10·0%).

Degradation of Morphine from (±)-[3-^{14}C]*Reticuline Feeding.*—Radioactive morphine (relative molar activity, 1·00), obtained from the (±)-[3-^{14}C]reticuline feeding experiment reported [14] earlier, was converted, by the usual methods, into codeine methiodide (relative activity, 0·99) and thence into α-codeimethine (relative activity, 0·99). This methine (238 mg.), in methanol 3 ml., was heated under reflux with methyl iodide (1 ml.) in the dark for 3·5 hr. Evaporation afforded α-codeimethine methiodide [30] (317 mg.), m. p. 254—256° (relative activity, 0·97). The methiodide (307 mg.) in water (3 ml.) and methanol (10 ml.) was shaken with freshly precipitated silver oxide (from 1 g. silver nitrate) until the solution was free from halide. After filtration, the solution (30 ml.) was treated with potassium hydroxide (30 g.) under reflux for

[29] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J.*, 1964, 1595.

[30] S. B. Schryver and F. H. Lees, *J.*, 1901, 563; L. Knorr, H. Hörlein, and C. Grimme, *Ber.*, 1907, **40**, 3844.

4 hr. and then extracted with benzene. The extract was washed with dilute hydrochloric acid and water and then dried and evaporated to leave methylmorphenol [31] (80 mg.), m. p. 61—63°, Sublimation (100°/0·2 mm.) gave material, m. p. 64—65°, having negligible radioactivity (relative activity, 0·027).

Extraction and Separation of Alkaloids.—Thirty *P. somniferum* plants (variety Noordster) were fed with (±)-[3-^{14}C]norlaudanosoline hydrochloride (60 mg., 0·43 mc) and harvested after periods of 36 hr. (3 plants), 48 hr. (9 plants), 58 hr. (8 plants), 72 hr. (7 plants), 82 hr. (3 plants), and 96 hr. (3 plants). Each batch of plants was worked up separately by maceration with aqueous acetic acid (5% by vol.) in a Waring blender and the suspension was then poured into a glass column. Aqueous acetic acid (5% by vol.; 2—3 l. per plant) was percolated through the macerated material. Approximately 10% of the percolate from each batch was reserved for future studies while the remaining aqueous extracts were combined and evaporated to *ca.* 200 ml. The concentrate was diluted with water (800 ml.), adjusted to pH 1·0 with phosphoric acid, and then extracted with ether (5 × 1 l.). Each ethereal extract was back-washed with aqueous phosphoric acid (2% by vol.; 50 ml.). The combined aqueous solutions were adjusted to pH 8·5, extracted with 4 : 1 (by vol.) chloroform–propan-2-ol (6 × 200 ml.) and the combined extracts were shaken with 2N-sodium hydroxide (4 × 250 ml.), washed with water (2 × 150 ml.), dried, and evaporated, to give the non-phenolic alkaloids as a gum (650 mg.). The aqueous alkaline extracts were adjusted to pH 8·5 and extracted with 4 : 1 (by vol.) chloroform–propan-2-ol (5 × 300 ml.). Evaporation of the dried extracts gave the phenolic alkaloids as a gum (650 mg.).

The non-phenolic alkaloids were separated into a strongly basic fraction (257 mg.) and a weakly basic fraction (390 mg.) as previously described.[14] Thebaine (300 mg.) was added to the former, and the mixture, in benzene (4 ml.), was run on to a column of alumina (100 g.); elution was carried out with benzene–chloroform–propan-2-ol [32] (88·5 : 10 : 1·5). A trace of gum was eluted followed by thebaine (423 mg.) and then codeine (111 mg.). The isolated thebaine (423 mg.), in ethanol (4·0 ml.), was treated with picric acid (312 mg.) and the resulting precipitate of thebaine picrate (699 mg.) had m. p. 215—217° (decomp.) (from ethanol).

A solution of this picrate (699 mg.) in chloroform was applied to a column of alumina (20 g.) and elution was continued with chloroform until all the thebaine had been removed. Evaporation of the eluate gave thebaine (401·5 mg.), m. p. 190—192° (specific activity 3·61 μc/mmole).

Preparation of the [16-^{14}C]*Salutaridine.*—(*a*) *Dihydrothebaine-φ.* The foregoing thebaine (401 mg.) was reduced with sodium in liquid ammonia, essentially by the method of Bentley, Robinson and Wain,[17] to give dihydrothebaine-φ as a gum (399 mg.). A parallel run in which inactive thebaine was used gave a gum which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) as plates (360 mg.), m. p. 149—153° (lit.,[17] 154°).

(*b*) O-*Acetyldihydrothebaine-φ.* A mixture of the radioactive dihydrothebaine-φ, acetic anhydride (5 ml.), and anhydrous sodium acetate (0·3 g.) was heated at 100° for 45 min. The mixture was then evaporated to dryness and an excess of saturated sodium hydrogen carbonate solution was added to the residue. Extraction with ether (5 × 20 ml.) gave, on evaporation of the dried extracts, the product as a gum (447 mg.). A similar preparation using inactive thebaine also gave a gum which did not crystallise even after repeated chromatography. Mild alkaline hydrolysis of the gum regenerated dihydrothebaine-φ in good yield (m. p. and mixed m. p. 149—153°).

(*c*) [16-^{14}C]*Salutaridine.* The foregoing acetate (447 mg.) was processed as described earlier, to give salutaridine (140 mg.). This, in ethanol (2 ml.), was treated with picric acid (100 mg.) and the precipitate was crystallised thrice from ethanol to give *salutaridine picrate* (206·7 mg.), m. p. 212—216° (decomp.) (Found: C, 54·0; H, 4·35. $C_{25}H_{24}N_4O_{11}$ requires C, 54·1; H, 4·45%).

All this picrate in methylene dichloride (10 ml.) was percolated over alumina. Chloroform eluted the main fraction which was crystallised twice from methanol to give [16-^{14}C]salutaridine (100 mg.), m. p. 197—199°; the specific activity (3·59 μc/mmole) was unchanged by the second crystallisation.

[16-^{14}C]*Salutaridinol-I and* [16-^{14}C]*Salutaridinol-II.*—Salutaridine (80 mg.) in methanol (5 ml.) was treated portionwise during 1 hr. with sodium borohydride (500 mg.). After a further 90 min. the solution was evaporated. The residue in water (10 ml.) was saturated with carbon dioxide and then extracted with chloroform (5 × 25 ml.). The combined extracts

[31] L. Knorr, *Ber.*, 1889, **22**, 181; E. Vongerichten, *ibid.*, 1896, **29**, 65.
[32] H. Rapoport, F. R. Stermitz, and D. R. Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 2765.

were washed with water (2 × 10 ml.), dried, and evaporated, and the residue, in chloroform (2 ml.), was applied to an alumina column (25 g.) which was eluted with chloroform–ethyl acetate (1 : 1 by vol.), chloroform, and chloroform–methanol (98 : 2 by vol.) in that order. The first compound eluted was [16-^{14}C]salutaridinol-I which crystallised from methanol as needles (29·5 mg.), m. p. 220—222° (decomp.).

The second compound eluted, [16-^{14}C]salutaridinol-II, was obtained as a clear glass (33·4 mg.) after further purification over alumina; it was shown to be homogeneous by thin-layer chromatography.

A similar reduction of inactive salutaridine with sodium borohydride, using dry dimethyl sulphoxide as the solvent, yielded salutaridinol-I, m. p. and mixed m. p. 220—222°, as the only isolable product.

[7-^{3}H]*Salutaridinols-I and -II.*—A suspension of finely powdered inactive salutaridine (100 mg) and sodium [^{3}H]borohydride (0·2 mg.) in dry propan-2-ol (1·5 ml.) was kept at 40° for 3 hr. Inactive salutaridinols-I and -II (10·0 mg. each) were then added and the bases were isolated as described in the foregoing experiment. These were dissolved in the minimum volume of chloroform and applied to an alumina column (25·0 g.) which was eluted with ethyl acetate–chloroform (1 : 1), chloroform, and chloroform–methanol (98 : 2) in that order, to give fractions containing dienone, dienone and dienol-I, dienol-I, and [7-^{3}H]salutaridinol-II (13·4 mg.). The second and third fractions were combined and rechromatographed to give [7-^{3}H]salutaridinol-I (12·0 mg.). The tritiated salutaridinols were each dissolved in 2N-sodium hydroxide (3·5 ml.; saturated with nitrogen) and kept at room temperature for 3 hr. and worked up separately as follows. The solution was saturated with carbon dioxide, extracted with chloroform (6 × 10 ml.), and the water-washed extracts were evaporated; an aliquot was removed for radioactive assay. This treatment with aqueous alkali was repeated once more with each dienol; the specific activities were unchanged.

[7-^{3}H,16-^{14}C]*Salutaridinols-I and -II.*—The ^{14}C-, and ^{3}H-labelled salutaridinols-I prepared above were combined and crystallised thrice from methanol to give the [7-^{3}H,16-^{14}C]salutaridinol-I as needles (32·9 mg.), m. p. 220—222° (decomp.), which did not change in specific activity after the second crystallisation (specific activity 2·60 μc ^{14}C/mmole; 38·2 μc ^{3}H/mmole).

Similarly, the ^{14}C- and ^{3}H-labelled salutaridinols-II were combined and chromatographed on alumina (10 g.) twice to give [7-^{3}H,16-^{14}C]salutaridinol-II as a glass (40·4 mg.) of constant specific activity (specific activity 2·82 μc ^{14}C; 32·6 μc ^{3}H).

Conversion of Salutaridinols-I and -II into Thebaine.—Samples of inactive dienol-I (10 mg.) and dienol-II (27 mg.) were dissolved in separate portions of 0·2M-phosphate buffer solution at pH 4·0 (10 ml.) and kept at room temperature for 60 hr. Each sample was then made strongly alkaline and extracted with chloroform (5 × 10 ml.). The combined, dried extracts from each sample were evaporated, and the residues purified by sublimation at 150°/10^{-4} mm. to give thebaine, identified by m. p. and mixed m. p. 191—193°.

A small sample of tritiated salutaridinol-I of accurately known total activity was dissolved in 0·2M-phosphate buffer at pH 5·3 (4·0 ml.) and kept at room temperature for 94 hr. The non-phenolic material was extracted as described above for a determination of its activity. A sample of tritiated salutaridinol-II was identically treated. It was demonstrated by control experiments that tritiated salutaridinols-I and -II are quantitatively removed from thebaine by the above procedure.

The conversion rate of salutaridinol-I into non-phenolic compounds was 0·176%/hr., and that of salutaridinol-II was 0·130%, the ratio of these rates being 1·35.

Cultivation of Papaver somniferum *Plants and Administration of Labelled Precursors.*—For general directions see Parts II [33] and IV.[14] A solution of [16-^{14}C]salutaridine (19·7 mg.; 0·215 μc) in 0·125M-phosphate buffer (4·5 ml. initially of pH 4·05) at pH 6·5 ± 0·5 was injected into 3 plants. In a similar manner solutions of salutaridinol-I (0·255 μc ^{14}C; 3·75 μc ^{3}H; 32·5 mg.) and salutaridinol-II (0·341 μc ^{14}C; 3·94 μc ^{3}H; 40·0 mg.) were prepared (pH 6·5 ± 0·5) and injected into two groups of 3 plants. One plant from each group was harvested after 1·5 days, a second after 2·5 days, and the third after 4 days. The plants were deep-frozen until required for extraction.

Extraction and Separation of Alkaloids. (*a*) The *P. somniferum* plants which had been fed with [7-^{3}H,16-^{14}C]salutaridinol-I were macerated with methanol (1 l.) in a Waring blender, packed into a glass column, and percolated with methanol (9 l.). The percolate was evaporated

[33] A. R. Battersby and B. J. T. Harper, *J.*, 1962, 3526.

and the residue was rapidly partitioned between ethyl acetate (250 ml.) and 0·5M-phosphate buffer at pH 5·3. After the two phases had been separated, the organic layer was further extracted with buffer solution (6 × 150 ml.). The combined aqueous extracts were washed with ether (5 × 100 ml.) and the extracts were back-washed with buffer solution (3 × 100 ml.). The combined aqueous solutions were made strongly alkaline with potassium hydroxide and extracted with chloroform (7 × 200 ml.); the aqueous phase *A* was reserved. After washing the chloroform extracts with 2N-sodium hydroxide (2 × 100 ml.) and water (3 × 200 ml.), they were dried and evaporated to give the non-phenolic alkaloids (40·1 mg.). Inactive codeine (94·0 mg.) and inactive thebaine (46·4 mg.) were added to this residue and the resultant mixture was fractionated as described above.

The isolated thebaine fraction (57·6 mg.) in ethanol (1·5 ml.) was treated with picric acid (42·7 mg.) and the picrate was mixed with inactive thebaine picrate (20 mg.) and recrystallised four times from ethanol to give thebaine picrate (97·3 mg.), m. p. 214—216° (decomp.). This was decomposed on an alumina column as previously described to give thebaine (55·7 mg.), m. p. 190—192°, which did not change in activity upon crystallisation from aqueous ethanol.

The codeine fraction (104·2 mg.) in ethanol (2 ml.) was treated with picrolonic acid (92 mg.) and the precipitate was mixed with inactive codeine picrolonate (41·4 mg.) and then recrystallised twice from ethanol to give codeine picrolonate (174 mg.), m. p. 230—232° (decomp.). Decomposition of the picrolonate by the method described for the decomposition of thebaine picrate gave codeine (106·6 mg.), m. p. 153—155°, which did not change in activity upon crystallisation from aqueous ethanol.

The aqueous phase *A* above was worked up for morphine as described [14] in Part IV and after the addition of inactive morphine (30 mg.) afforded morphine hydrochloride (125·5 mg.). This was dissolved in ethanol, treated with picric acid (100 mg.), and the precipitate was crystallised thrice from ethanol to give morphine picrate (128 mg.), m. p. 160—161° (decomp.). The picrate was dissolved in ethanol (1·0 ml.), water (3·0 ml.), and N-hydrochloric acid (3·0 ml.), and the solution was extracted continuously with ether until the extracts were colourless. The aqueous phase was evaporated to dryness and the residue in water (1·0 ml.) was treated with a slight excess of sodium hydrogen carbonate to afford morphine hydrate (71·2 mg.), m. p. 250—253° (decomp.), of constant specific activity.

(*b*) The plants fed with [7-^{3}H,16-^{14}C]salutaridinol-II were worked up as described under (*a*) to give thebaine (55·4 mg.), m. p. 191—193°, codeine (70·0 mg.), m. p. 154—156°, and morphine hydrate (75·4 mg.), m. p. 252—255° (decomp.), all of constant specific activity.

A solution of the phenolic alkaloids together with inactive salutaridine (17·5 mg.) in propan-2-ol (1 ml.) and chloroform (5 ml.) was run on to a Fluorosil column (20 cm. × 2 cm.) which was eluted with chloroform (100 ml.), chloroform–methanol (20 ml.) (95 : 5 by vol.), chloroform–methanol (170 ml.) (9 : 1 by vol.), and methanol–acetic acid (500 ml.) (95 : 5 by vol.) in that order. The residue from the chloroform–methanol eluates was applied, in chloroform, to an alumina column (10 g.) and elution was continued with ethyl acetate–benzene (4 : 1 by vol.) to give crude salutaridine (23·0 mg.). This was diluted with inactive salutaridine (20 mg.) and purified as the picrate; regeneration of the free base gave pure salutaridine as needles (38·9 mg.), m. p. and mixed m. p. 197—199°.

Evaporation of the acetic acid–methanol eluates above left a residue which was dissolved in water, and the solution was adjusted to pH 8·3 before extraction with chloroform–propan-2-ol (4 : 1 by vol.) (6 × 15 ml.). The crude morphine obtained by evaporation of these extracts was purified as previously described to give morphine hydrate (122·3 mg.), m. p. 255—257° (decomp.), of constant specific activity.

Isolation of Salutaridine.—Part (3/16 of total) of the phenolic alkaloids from the "rate-feeding" of [3-^{14}C]norlaudanosoline was diluted with inactive salutaridine (31·7 mg.) and the appropriate fraction (30·3 mg.) was isolated by chromatography on Fluorosil as previously described. This was purified as the picrate and the pure salutaridine was recovered from the picrate by pouring a chloroform solution through a column of alumina. The pure salutaridine (23·1 mg.), m. p. 197—199°, showed 3·4 × 10^{3} dis./100 sec./mg. Incorporation, 0·034%.

Conversion into Thebaine.—The foregoing active salutaridine (15 mg.) was diluted with inactive salutaridine (15 mg.) and a solution of the mixture in methanol (5 ml.) was reduced with sodium borohydride (150 mg.). The mixture of dienols so obtained was dissolved in 0·5M-phosphate buffer (10 ml.; pH 2·9) and kept at room temperature for 5 days. The solution was extracted thrice with chloroform, then was basified strongly with sodium hydroxide and again

extracted with chloroform. The latter extracts were washed with water, dried, and evaporated, to give a gum (20 mg.). This was converted into the picrate which was recrystallised four times from ethanol to give pure thebaine picrate (22 mg.), m. p. 215—217° (decomp.). The recovered thebaine (as above) was sublimed at 170°/10^{-4} mm. to give pure thebaine (12·7 mg.), m. p. 190—192°. The diluted salutaridine had specific activity of $1{\cdot}51 \times 10^{-1}$ μc/mmole and the thebaine had specific activity of $1{\cdot}44 \times 10^{-1}$ μc/mmole (ratio 1·00 : 0·95).

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