which is stabilized further by hydrogen bonding and by van der Waals attractions is possible. The relatively low yield of purified product may be considered as further evidence for such a complex.

This work was carried out with corticotropin of beef origin. Beef glands contain a much lower concentration of corticotropin than pork, and best results were obtained using methanol–acetic acid extraction of defatted glands[5] for the preparation of the crude acid extract which was usually obtained at a potency of 0.8–1.2 U.S.P. units per mg. Rapid freezing of the glands after removal was necessary for good results. The yield and potency of corticotropin from various sources is shown in Table I.

TABLE I

YIELD AND POTENCY OF CORTICOTROPIN FROM 1 KG. OF FRESH GLANDS

Source	Defatted gland, wt. (g.)	Crude acid extract Wt. (g.)	Crude acid extract Potency, μ/mg.	Oxycellulose purified Wt. (mg.)	Oxycellulose purified Potency, μ/mg.	Ref.
Sheep	..	..	.....	200	30–50	4
Pork	150	18	2–3	450	84	5
Beef	185	35	0.7–1.2	700	20–30	

Although beef glands yield corticotropin of lower potency and in smaller quantity than either sheep or pork glands, the yield and potency are such that this source of corticotropin could be utilized if necessary. Although beef corticotropin purified by the oxycellulose procedure is undoubtedly different in some aspects from either sheep or pork corticotropin,[4] the applicability and specificity of the cellulose procedures with corticotropin from these sources is evidence that basic groups of similar pK and spatial arrangement are present in each of these preparations. That these groups are identical and a requisite of activity is a further, but more speculative, conclusion.

TABLE II

FRACTIONATION OF BEEF CORTICOTROPIN USING CELLULOSE DERIVATIVES

Sample	Adsorbent	Cellulose derivative, mg. per g. of crude	Stirring time, hr.	Acid extract,[a] μ/mg.[b]	Purified product, μ/mg.	Activity recovery, %
Lot 1	Oxycellulose	193	4	0.75	18	52
	Cellulose acid citrate[6]	250	4	.75	20	44
	Oxycellulose	200	2	.75	25	46
	Oxycellulose	161	24[c]	.75	20	57
Lot 2	Cellulose acid citrate	250	3.5	1.1	30	46
	Oxycellulose	114	2.5	1.1	30	19
	Cellulose acid phosphate[6]	240	3.5	1.1	14	20
Lot 3	Oxycellulose	250	5	0.8	15	..
	Cellulose acid citrate	500	6	.8	11	29
Lot 4	Carboxymethyl cellulose[7]	50	20[d]	.75	19	5

[a] The acetic acid extract was prepared by the method described in ref. 5. [b] U.S.P. units, modified ascorbic acid depletion method.[8] [c] No Tween 20 added. [d] The sodium salt was converted to the free acid form prior to use. The activity was eluted with ammonium hydroxide.

(6) Kindly supplied by Tennessee Eastman Co.

(7) Received from P. D. Weildon, Hercules Powder Co. The product was rendered insoluble in water by partial internal lactonization which was obtained by drying this material at 100° before use.

The experimental procedure consisted in slurrying a dilute solution of the crude acid extract with the cellulose derivative being studied for 2–4 hours in the presence of a surfactant. Using 0.1% of a non-ionic polyoxyethylene sorbitan derivative (Tween-20) the potency and yield of product was similar to that obtained by the published procedures in which a slurry time of 24–40 hours is used. The results obtained with the various cellulose derivatives are shown in Table II.

Experimental

Preparation of Defatted Gland Tissue.—Frozen beef pituitary glands (1 kg.) were partially thawed and ground using a Hobart meat grinder. The ground tissue was slurrried with 4 l. of acetone. The slurry was allowed to settle and the upper phase decanted. The solid residue was slurried and decanted with two additional 2-l. portions of acetone followed by three 2-l. portions of methanol and finally filtered, washed with ether and dried yielding 185 g. of defatted gland tissue. This material may be stored at room temperature for reasonable periods with no detectable loss of activity.

Preparation of Crude Acid Extract.—Defatted beef gland tissue (100 g.) was stirred with 900 ml. of methanol and 600 ml. of acetic acid at reflux temperature for two hours in a closed apparatus protected from moisture by a drying tube. At the end of this time the mixture was centrifuged and the decantate collected. The residue was washed with 40% acetic acid–methanol and the washings combined with the decantate. The crude acid extract was obtained by adding an equal volume of anhydrous ethyl ether to the decantate, filtering, washing with ether and drying, wt. 35 g., potency 0.7–1.2 u. per mg.

Cellulose Purification.—Crude corticotropin was dissolved in sufficient water to make a 2.5% solution. Tween-20 (1 mg. per ml.) was added and the mixture slurried at room temperature with the required amount of cellulose derivative (usually 20–25% of the weight of the crude) using a mechanical stirrer for 2–4 hours. The mixture was filtered and washed with 0.1 N acetic acid until the washings gave a negative biuret test taking care to slurry the mixture well with each wash. Usually three or four washes of 10 ml. for each gram of cellulose derivative were required. The washed adsorbate was slurried for one-half hour with two successive portions of 0.1 N hydrochloric acid (10 ml. total per g. of adsorbate). The combined eluate was neutralized to pH 3.0 by slurrying with strong base ion exchange resin (such as IRA-400) as the carbonate and lyophilized. The dried products are stable at room temperature when stored under moisture free conditions.

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(8) N. G. Brink, F. A. Kuehl, Jr., M. A. P. Meisinger, M. N. Bishop and K. Folkers, THIS JOURNAL, **74**, 480 (1952).

CHEMICAL DIVISION
MERCK AND COMPANY, INC.
RAHWAY, NEW JERSEY

A Method for Preparing Codeinone

BY HENRY RAPOPORT AND HELEN N. REISI

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In the course of preparing various glycosides of codeine[1] for biological investigations, the consistently poor yields induced us to examine the

(1) P. Casparis, E. Kühni and E. Leinzinger, *Pharm. Acta Helv.*, **24**, 145 (1949).

reaction in greater detail. The procedure being employed was the usual treatment of a benzene solution of an alcohol (codeine) with an acetylglycosyl bromide in the presence of silver carbonate. As the reaction proceeded, there was a pronounced frothing and the silver carbonate became black. Analysis of the benzene-insoluble material at the conclusion of the reaction established the presence of considerable free silver, indicating that an unexpected oxidation had occurred. That it was the codeine being oxidized was demonstrated by an experiment in which the acetylglycosyl bromide was omitted and from which codeinone was isolated in fair yield.

A study was then made of various factors in this reaction and their effect on the yield of codeinone. The quantity of silver carbonate, the mode of addition of silver carbonate and codeine, and the time of reflux were all varied, and conditions were found which resulted in a 75% yield of codeinone. These consisted of simply heating under reflux a solution of codeine in benzene with 500 mole per cent of silver carbonate. Although the chromic acid oxidation of codeine to codeinone has been much improved recently[2] and an excellent Oppenauer oxidation procedure is now available,[3] the simplicity and very good yield of the present silver carbonate method make it an attractive alternative for preparing codeinone.

A search of the literature revealed no such previous application of silver carbonate, although silver oxide in anhydrous ether has been used in the preparation of quinones[4,5] and silver salts (and silver hydroxide) have served for various oxidations.[5] In seeking possible extensions and limitations of this silver carbonate oxidation procedure, the reactions with dihydrocodeine and neopine were tried. No reaction took place with dihydrocodeine and it was recovered quantitatively. With neopine definite blackening of the silver carbonate occurred but the only isolable product was neopine in an 18% recovery.

Experimental

Preparation of Silver Carbonate.—The yellow precipitate of silver carbonate formed when 300 ml. of an aqueous solution containing 25.6 g. of sodium bicarbonate was added to 480 ml. of 10% aqueous silver nitrate was washed by decantation six times with 1.5-l. portions of distilled water and four times with 700 ml. portions of methanol. It was then transferred to and washed on a suction filter funnel using a total of 2 l. of absolute ether. After being sucked dry on the funnel for about 20 min., the product was stored *in vacuo* over magnesium perchlorate in the dark. When first prepared, the silver carbonate is yellow, but on storage the color gradually changes to yellow-green and finally brown. This seems to have no adverse effect on its ability to oxidize codeine, and preparations over a month old have been used successfully.

Anal. Calcd. for Ag_2CO_3: Ag, 78.2; C, 4.4. Found: Ag, 78.1; C, 4.4.

Codeinone.—After 25 ml. was distilled from a solution of 6 g. (0.02 mole) of codeine in 125 ml. of benzene, 27.6 g. (0.1 mole) of silver carbonate was added and the mixture was heated under reflux with rapid stirring in a nitrogen atmosphere for one hour during which an additional 10 ml. of solvent was removed by distillation. The hot mixture was then filtered, the insoluble portion was digested with two 50-ml. portions of benzene, and the combined filtrate and digests were concentrated at the water-pump until crystals began to appear. These were removed by filtration after cooling, and the filtrate was further concentrated. In this manner, two crops were obtained, for a total of 4.5 g. (75% yield), melting at 179–182° to a characteristic red melt. On recrystallization from benzene using decolorizing carbon, 4.0 g. of practically colorless codeinone resulted, m.p. 181–182°, $[\alpha]^{21}D$ −208° (*c* 1.0, 95% ethanol) [reported[2] m.p. 181.5–182.5°, $[\alpha]^{20}D$ −205° (*c* 0.8, 99% alcohol)].

Codeinone oxime hydrochloride was prepared and melted at 257–260° dec. (reported[2] m.p. 258°). On reduction with sodium borohydride, codeinone was converted to codeine,[6] m.p. 156–157°.

(2) S. P. Findlay and L. F. Small, THIS JOURNAL, **72**, 3247 (1950).

(3) A. H. Homeyer and G. B. DeLaMater, U. S. Patent 2,654,756 (Oct. 6, 1953). Using this process we have obtained repeated yields of 40–60% of codeinone.

(4) R. Willstätter and A. Pfannstiel, *Ber.*, **37**, 4744 (1904).

(5) *E.g.*, J. Houben, "Die Methoden der Organischen Chemie," Third Edition, Vol. 2, Verlag Georg Thieme, Leipzig, 1925.

(6) M. Gates, THIS JOURNAL, **75**, 4340 (1953).

DEPARTMENT OF CHEMISTRY AND RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

On the Role of Polyphenoloxidase in Lignin Biosynthesis

BY H. S. MASON AND M. CRONYN

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It has been proposed that lignin is formed from coniferyl alcohol[1] under the influence of polyphenoloxidase and oxygen.[2–7] However, in the experiments upon which this hypothesis is based, the enzyme preparations which were employed were crude and presumably heterogeneous[8]; and conditions were utilized in which the growth of microorganisms[9] as well as the denaturation of enzymes is favored.[10]

Our interest in this matter derives from the possibility that polyphenoloxidases have continuity of biochemical function throughout the plant and animal worlds and that the lignins correspond to some pigments of higher organisms in being the products of the action of polyphenoloxidases upon *o*-diphenols (*cf.* reference 13).

Accordingly, we have re-examined the action of mushroom polyphenoloxidase, prepared by following the directions of Freudenberg,[2] upon coniferyl alcohol, and have compared it with the corresponding action of a purified mushroom polyphenoloxidase. Typical results are depicted in the Fig. 1. Oxygen consumption by coniferyl alcohol in the presence of Freudenberg crude oxidase was equivalent to about $^1/_2$ atom per molecule, an observa-

(1) P. Klason, *Svensk. Kem. Tidsk.*, **9**, 135 (1897); cited by G. de Stevens and F. F. Nord, *Proc. Nat. Acad. Sci.*, **39**, 80 (1953).

(2) K. Freudenberg and H. Richtzenhain, *Ber.*, **76**, 997 (1943).

(3) K. Freudenberg, H. Resnik, H. Beosenberg and D. Rasenack, *ibid.*, **85**, 641 (1952).

(4) K. Freudenberg and W. Heimberger, *ibid.*, **83**, 519 (1950).

(5) K. Freudenberg and H. Dietrich, *ibid.*, **86**, 1157 (1953).

(6) K. Freudenberg and F. Bittner, *ibid.*, **86**, 155 (1953).

(7) L. Freudenberg, R. Kraft and W. Heimberger, *ibid.*, **84**, 473 (1951).

(8) The enzyme was variously described by Freudenberg as catecholoxidase, phenoldehydrogenase, mushroom dehydrogenase and redoxase.

(9) These conditions involved incubations of 3–28 days in the presence of crude mushroom proteins.

(10) Oxygen was continuously bubbled through the reaction mixtures. This has been shown to inactivate mushroom polyphenoloxidase.[11-13]

(11) M. H. Adams and J. M. Nelson, THIS JOURNAL, **60**, 2474 (1938).

(12) I. Asimov and C. R. Dawson, *ibid.*, **70**, 1184 (1948).

(13) G. Johnson and L. A. Schaal, *Science*, **115**, 627 (1952).