acid catalyzed decarboxylation was investigated under the same conditions as the acid catalyzed dehydration.

A mathematical treatment was developed to handle a system of reactions of the type A $\rightleftarrows$ B $\longrightarrow$ C, where the reaction rate constants are comparable.

The acid catalyzed hydration, dehydration and decarboxylation reactions are first order with respect to the organic acid concentration and first order with respect to the hydrogen ion concentration.

The reaction rate constants of these three reactions increase rapidly with ionic strength and slowly when sodium ion replaces hydrogen ion at constant ionic strength.

The uncatalyzed decarboxylation of β-hydroxyisovaleric acid in distilled water and in aqueous sodium perchlorate solutions was investigated at 99.85 and 111.85°. The reaction is first order with respect to the organic acid. The constant is somewhat smaller than the constant for the acid catalyzed decarboxylation, and increases moderately with ionic strength. Decarboxylation involves the free acid and not the anion.

The rate of decarboxylation of β,β-dimethylacrylic acid in pure water is negligible compared to that of β-hydroxyisovaleric acid.

The heat of activation of the hydration of β,β-dimethylacrylic acid essentially is not a function of ionic strength or hydrogen ion concentration.

For the hydration of dimethylacrylic acid, ΔH is −8.1 kcal. The correct value for crotonic acid is −5.4 kcal.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE, AND THE GEORGE WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

A Simple Laboratory Method for Obtaining Preparations Containing Pressor and Oxytocic Activity from the Posterior Lobe of the Pituitary Gland

BY GEORGE W. IRVING, JR.,[1] AND VINCENT DU VIGNEAUD

The observation recorded in a previous paper[2] that practically all of the pressor and oxytocic activity contained in posterior lobes could be removed in the press juice obtained by subjecting the ground glands to high pressure in a hydraulic press, suggested to us that this method might be utilized for the extraction of the principles from glands on a preparative scale. Dry-ice frozen posterior lobes were thawed, ground with sand and subjected to pressure in a hydraulic press. The juice obtained, together with several washes of the sandy residue, was acidified with acetic acid. The mixture was heated for ten minutes in a boiling water-bath to precipitate inactive protein. The clear solution obtained by centrifugation was concentrated by evaporation in a stream of clean air at room temperature. The active material was precipitated from the concentrated solution by saturation with sodium chloride as described by Kamm and his associates.[3] The dried salt-cake was extracted with 98% acetic acid, and the active material was precipitated from the acetic acid solution by means of ether and petroleum ether. This final product, which will be referred to as "ether precipitate," is a chalk-white, non-hygroscopic, water-soluble powder. It contains from 9 to 10 units of pressor and oxytocic activity per mg.

A comparison of the yield of active material obtained by the above procedure with the yields recorded in the literature for the extraction of acetone desiccated glands, shows that the present method compares favorably with previous procedures. Both Kamm, *et al.*,[3] and Stehle and Fraser[4] report that commercial acetone desiccated posterior lobes contain 1 unit of each activity per mg. They were able to obtain from this material a crude product which contained 80 to 90% of the initial activity. On this basis 1 kg. of fresh glands (approximately 160 g. of acetone desiccated powder) would yield by the procedures of Kamm, *et al.*, or Stehle and Fraser, a crude preparation containing about 135,000 units of each activity. By the procedure outlined in this paper, 1 kg. of frozen glands yields 13 g. of ether precipitate

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(2) Irving and du Vigneaud, *J. Biol. Chem.*, **123**, 485 (1938).

(3) Kamm, Aldrich, Grote, Rowe and Bugbee, THIS JOURNAL, **50**, 573 (1928).

(4) Stehle and Fraser, *J. Pharmacol.*, **55**, 136 (1935).

which contains approximately 120,000 units of each activity.

As a further check upon the efficiency of the present method the following experiment was performed. A batch of 850 g. of frozen posterior lobes was divided into two equal portions. One portion was treated according to the procedure outlined in this paper. The other portion was treated according to the directions of Kamm and his associates.[3] By the present method, 7.6 g. of ether precipitate which contained 74,500 pressor and 76,800 oxytocic units was obtained. By the procedure of Kamm, *et al.*, 8.6 g. of a product which contained 80,000 pressor and 90,300 oxytocic units was obtained. Within the error of assay, these yields may be considered comparable.

Experimental

Preparation of the Active Extract.—The procedure to be given is described for 450 g. lots of beef or hog posterior lobes. The posterior lobes were received from Detroit, Michigan,[5] in containers which were surrounded by sufficient dry-ice to keep the material solidly frozen during shipment.

The frozen glands are partially thawed and the mass is thoroughly ground in a porcelain mortar with an equal weight of clean, white sand. Two hundred cc. of distilled water is added and the mixture is homogenized by further grinding. The "juice," which contains the active principles, is separated by subjecting the ground mixture to approximately 20,000 pounds pressure in the $2^1/_4$ inch (5.8 cm.) test cylinder of a Carver Laboratory Press.[6] This fluid (500 cc.) is collected in an ice-cooled flask. It is a viscous, frothy liquid which contains from 60 to 70% of the pressor and oxytocic activities initially present in the glands. The sandy residue is ground with 200 cc. of distilled water and the wash liquid is expressed. This process is repeated three more times. The wash fluid (approximately 750 cc.) contains an additional 20 to 30% of the active material initially present in the glands. The final residue contains only 1 to 2% of the active material and may be discarded.

The juice is diluted (1 volume of juice plus 2 volumes of distilled water) and is acidified with glacial acetic acid (0.5 cc. per 100 cc. of diluted juice). Acidification results in the separation of a voluminous precipitate. The suspension is thoroughly mixed and is heated for ten minutes in a boiling water-bath with frequent mixing. The wash is acidified and treated in a similar manner without dilution. For the heat treatment the acidified material is distributed in 300-cc. portions among several 500-cc. Erlenmeyer flasks. After being rapidly cooled to room temperature the precipitated inactive protein is removed by centrifugation. The clear, straw-colored supernatant, which contains the active principles, is poured off and the precipitant is washed with two successive 150-cc. portions of cold 0.25% acetic acid. The washed precipitate contains only a negligible amount of activity and can be discarded. The main extract and the dilute acetic acid wash of the heat precipitate are combined and the mixture is further acidified by adding 0.2 cc. of glacial acetic acid for each 100 cc. of extract. The entire solution (about 2000 cc.) is then poured into a shallow Pyrex dish and is placed before a fan. Evaporation to 400 to 500 cc. takes place in thirty-six to forty-eight hours. To prevent contamination of the evaporating solution by dust from the air the Pyrex dish and fan can be enclosed in a wooden box, the open ends of which are covered by several layers of cheesecloth.

The distribution of active material in the various fractions in the foregoing procedure is summarized in Table I. The juice and wash fractions were assayed directly for both pressor and oxytocic activity.[7] The sandy residue and the washed heat-precipitate were extracted exhaustively with hot 0.25% acetic acid and this extract was assayed for both principles. The sum of these assays was taken as 100% and the percentages given in Table I were calculated on this basis. By the procedure up to this point approximately 90% of both principles has been obtained in the form of an aqueous extract directly from the glands.

TABLE I

DISTRIBUTION OF PRESSOR AND OXYTOCIC ACTIVITY IN THE VARIOUS FRACTIONS

Fraction	Activity, % Pressor	Activity, % Oxytocic
Expressed juice	70	60
Expressed wash	20	35
Sandy residue	2	1
Heat precipitate	8	4

Preparation of the Salt-cake.—The concentrated extract is diluted, if necessary, to 500 cc. with 0.25% acetic acid and 150 g. of sodium chloride is added slowly with vigorous mixing. The mixture is allowed to stand overnight in the refrigerator and the precipitate is filtered off. The salt-cake is sucked as dry as possible on the filter and is then dried over phosphorus pentoxide in a vacuum desiccator. The mother liquor, which contains only traces of activity, is discarded.

The salt-cake is a cream colored, non-hygroscopic powder having a pressor and oxytocic potency of approximately 5 units per mg. The salt-cake weighs approximately 15 g. and it contains 95 to 100% of the active material present in the extract.

Preparation of the Ether Precipitate.—The pulverized salt-cake is extracted with 98% acetic acid by the procedure outlined by Kamm, *et al.*,[3] in the footnote, p. 589. The acetic acid solution (approximately 100 cc.) is treated with vigorous shaking, with 2.5 volumes of anhydrous, peroxide- and aldehyde-free ethyl ether, followed immediately by 5 volumes of anhydrous Skellysolve B. The white precipitate is allowed to settle for about one hour in the refrigerator and is then filtered off by means of gentle suction.

(5) We wish to express our appreciation to Dr. Oliver Kamm of Parke, Davis and Co. for generous supplies of frozen glands.

(6) For this step in the procedure a more satisfactory apparatus, with a larger capacity, has been devised. A description of the improved juice extractor can be found in: Irving and Loring, *Ind. Eng. Chem., Anal. Ed.*, **32**, *in press* (1940).

(7) Pressor assays were carried out on dogs under chloretone anesthesia, while oxytocic assays were made on hens under urethan as described by Sealock and du Vigneaud, *J. Pharmacol.*, **54**, 433 (1935).

It is washed on the filter with 300 cc. of a 2:1 mixture of petroleum ether and ether, and finally three times with ether. The washed product is dried in a vacuum desiccator over potassium hydroxide and the ether is removed by repeated evacuation. The yield from 450 g. of frozen glands is about 6 g.

A summary of the yields obtained from approximately 20 kg. of frozen posterior lobes by the method described is given in Table II. Each of the "lots" listed in the table represents a combination of the ether precipitate obtained from several 450 g. batches of posterior lobes. From the averages it can be seen that 1 kg. of frozen glands yields approximately 13 g. of a product which possesses a pressor and oxytocic potency of 9 to 10 units per mg. Approximately 120,000 units of each activity can therefore be obtained from one kg. of posterior lobes.

TABLE II

YIELDS OF ETHER PRECIPITATE AND PRESSOR AND OXYTOCIC ACTIVITY FROM FROZEN POSTERIOR LOBES

Lot	Frozen glands used, kg.	Ether ppt. per kg. of glands, g.	Activity per kg. of frozen glands: Pressor units	Oxytocic units
B	1.8	11.9	119,000	119,000
C	4.1	11.4	114,000	91,200
D	2.4	11.3	89,000	90,400
E	3.0	12.4	111,600	136,400
F[a]	5.9	13.2	118,800	145,200
G[b]	2.7	18.2	163,800	163,800
Averages		13.1	119,400	124,300

[a] Hog glands. All others are from beef. [b] The ether-precipitates included in Lot G were obtained from glands extracted by the use of the improved juice extractor.[6] All others were extracted using the 2¼-inch (5.8-cm.) test cylinder of the Carver press equipment.

The authors wish to express their appreciation to Mr. T. W. Loring for his assistance in this work.

Summary

A convenient laboratory method has been developed by which 80 to 90% of the pressor and oxytocic activities contained in frozen posterior lobes can be rapidly obtained in the form of a white, water-soluble, non-hygroscopic, stable powder possessing a pressor and oxytocic potency of 9 to 10 units per mg. In comparison with previous methods, desiccation of the glands with acetone and extraction of the residue are avoided; the volumes of solutions to be handled are small; and the expenditure of time, labor and solvents is less. The process can be used with equal efficiency to separate the active material from a few posterior lobes or from several kg. of glands.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE SCHOOL OF PHARMACY, UNIVERSITY OF MARYLAND]

Some Schiff Bases with *p*-Aminothymol[1]

BY W. TAYLOR SUMERFORD,[2] WALTER H. HARTUNG[3] AND GLENN L. JENKINS[4]

Introduction

The toxicity of acetanilide is attributed to its hydrolytic product, aniline, while the febrifuge and analgesic properties of acetanilide depend upon its conversion in the body to *p*-aminophenol.[5] The most useful substitutes for acetanilide have been the acyl derivatives of *p*-ethoxyaniline (*e. g.*, acetylphenetidin) which presumably act more safely by being slowly converted to *p*-aminophenol.

Since cresols are more active and less toxic than the corresponding phenols and the toluidines[6] are less toxic than aniline, it was thought that a compound hydrolyzable in the body directly to a mono- or a di-alkyl substituted *p*-aminophenol would be an antipyretic and analgesic lacking in toxicity.

The RN=CR linkage of a Schiff base[7] formed by the elimination of water from a primary amine and an aldehyde or ketone is subject to hydrolysis in an acid medium.

Salicylaldehyde, piperonal, and vanillin, as well as other carbonyl compounds, are known to have pharmacological properties in common with, and hence possibly augmentative to, those of *p*-aminophenol. Jacquet[8] has found that Schiff bases prepared by condensing physiologically active amines and aldehydes possess, at least in part, the pharmacological action of their components.

(1) Presented before the Medicinal Section of the American Chemical Society at the Cincinnati Meeting, April, 1940.

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(5) Barrowcliff and Carr, "Organic Medicinal Chemicals," Bailliere, Tindall and Cox, London, 1921, pp. 113–114.

(6) Bogert and Connitt, THIS JOURNAL, **51**, 900 (1929).

(7) Sidgwick, "Organic Chemistry of Nitrogen," revised by Taylor and Baker, Clarendon Press, Oxford, 1937, p. 65.

(8) Jacquet, *Pharm. Ztg.*, 613 (1893).