action mixture by distillation under vacuum on a steam-cone the residual material resisted all attempts at crystallization and its aqueous solution showed a specific rotation of less than one degree. Neuberger has reported[22] that acetyl-*l*-proline melts at 115° and that its aqueous solution shows a specific rotation of −104°. Fractional vacuum distillation of the aqueous solution gave 2.5 g. (80% yield) of acetyl-*dl*-proline, b.p. 146–153° (0.9 mm.).

Anal. Calcd. for $C_7H_{11}NO_3$: N, 8.92; neut. equiv., 157. Found: N, 8.71; neut. equiv., 165.

We wish to acknowledge the technical assistance of Messrs. Charles Anderson and Kenneth Hutton.

NEW YORK 11, N. Y.

(22) A. Neuberger, *Biochem. J.*, **32**, 1452 (1938).

[CONTRIBUTION FROM THE RESEARCH LABORATORY, DOMINION RUBBER CO., LTD.]

The Alkaloids of Fumariaceous Plants. XLVIII. The Structure of Corpaverine

BY RICHARD H. F. MANSKE

RECEIVED OCTOBER 25, 1951

An alkaloid (F24), now termed corpaverine, has been shown to be 2-methyl-6,7-dimethoxy-8-hydroxy-1-(4-methoxybenzyl)-tetrahydroisoquinoline. It is pointed out that it survives as a benzylisoquinoline in a plant which elaborates almost exclusively protoberberines because there is no activated position in the benzyl portion to permit further ring closure.

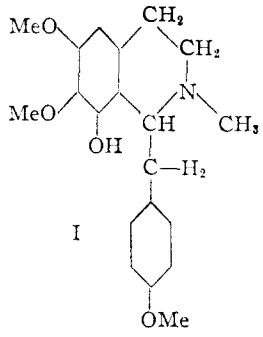
Alkaloid F24 has been isolated from *Corydalis aurea* Willd[1] in amounts less than one part per million. On mild oxidation it has yielded *p*-anisic acid and it is therefore obvious that this alkaloid, for which the name *corpaverine* is now proposed, is a benzylisoquinoline. Since it has four oxygen atoms—one phenolic hydroxyl and three methoxyls—its empirical formula must be $C_{20}H_{25}O_4N$ instead of $C_{19}H_{23}O_4N$ as previously given, the analytical figures being about equally good for both.

Evidently a number of *p*-methoxybenzylisoquinolines with two methoxyls and one hydroxyl in the isoquinoline nucleus are possible but bearing in mind the associated capaurine[2] it is quite evident that formula I is the most probable. When corpaverine was ethylated with diazoethane an O-ethyl ether was obtained which on treatment with methyl iodide yielded only a quaternary iodide. The nitrogen is therefore tertiary. This methiodide when degraded to the corresponding methine and the latter again methylated and heated with alkali yielded a neutral substance which on oxidation with permanganate yielded *p*-anisic acid and 3-ethoxy-4,5-dimethoxyphthalic acid. Corpaverine therefore has formula I if cognizance is taken of the fact that all natural trialkoxyisoquinolines have the substituents in the 6, 7 and 8-positions.

Corpaverine is of interest in connection with the biogenesis of the protoberberines. The plant in which it occurs is chiefly noted for the almost exclusive elaboration of protoberberines and the assumption that benzylisoquinolines are intermediates in their syntheses is virtually unavoidable. Such a last step however requires another ring closure and for this purpose a point of attack in a benzene ring must be activated by a hydroxyl or methoxyl in the ortho or para position. Such activation is lacking in corpaverine and in consequence this alkaloid is the end-product in the plant. It would be difficult to devise an experiment which would give clearer evidence of the nature of the ultimate intermediate in the biogenesis of the protoberberines.

(1) R. H. F. Manske, *Can. J. Research*, **B16**, 81 (1938).

(2) R. H. F. Manske and H. L. Holmes, THIS JOURNAL, **67**, 95 (1945).



I

Experimental

Owing to the extremely small amount of this alkaloid available, it was not possible to carry out the desirable isolation and characterization of the intermediates which were obtained in the various steps of its degradation.

Corpaverine is optically active; $[\alpha]^{20}D$ −154.2° (*c* 2.63 in chloroform).

Oxidation.—One hundred and fifty mg. of corpaverine was dissolved in 20 cc. of hot water to which had been added a small pellet of potassium hydroxide. The cooled solution was treated with small portions of potassium permanganate until the purple color remained permanent for 30 minutes. The decolorized solution was treated with calcium chloride, filtered, acidified, and extracted with ether. The residue from the ether extract was recrystallized twice from hot water and then consisted of colorless plates of *p*-anisic acid which melted sharply at 184°[3] either alone or in admixture with an authentic specimen.

Degradation.—A suspension of 0.4 g. of corpaverine in cold methanol was left for 24 hr. with an excess of an ethereal solution of diazoethane. The solvents were then removed from the clear solution and the residue dissolved in hot dilute hydrochloric acid. The filtered and cooled solution deposited colorless plates of a sparingly soluble hydrochloride which when recrystallized once from hot water melted at 247° when placed in the bath at 230°. The free *base* crystallized slowly from warm hexane in colorless plates and melted at 76°; calcd. for $C_{22}H_{29}O_4N$: C, 71.16; H, 7.81; N, 3.77. Found: C, 70.87; H, 7.70; N, 4.00. The methiodide separated as an oil when the base in ether was treated with excess methyl iodide. It dissolved only sparingly in hot water and separated as an oil on cooling the solution The addition of potassium hydroxide did not yield a turbidity in the cold. An excess of potassium hydroxide was added to the hot aqueous solution of the methiodide and the mixture heated for 12 hr. At intervals the separated methine was removed by extracting the cooled mixture with ether. The resulting methine was again converted to methiodide and the latter treated with an excess of silver oxide in hot aqueous solution. The filtrate from this mix-

(3) All melting points are corrected.

ture was heated on a steam-bath and the liberated oil extracted from the cooled solution at intervals. Trimethylamine was evolved during this decomposition. The resultant neutral product was dissolved in acetone, a drop of water added, and oxidized with potassium permanganate until the color of the added reagent was permanent for 30 min. Water was added, the acetone boiled out, and the mixture was filtered after a little calcium chloride had been added. The clear filtrate after acidification yielded to ether extraction a small amount of residue which was boiled with a little water and cooled. The sparingly soluble acid proved to be *p*-anisic acid. The more soluble fraction was treated with methylamine, evaporated to dryness, and the residue sublimed *in vacuo* (approx. 180° (0.5 mm.)). The sublimate was extracted with ether which left a small amount of insoluble material. The soluble portion was then recrystallized from hot water (m.p. 86°). It was again sublimed *in vacuo*, washed with pentane, and recrystallized again from water. It then melted at 89° and in admixture with an authentic specimen of 3-ethoxy-4,5-dimethoxy-N-methylphthalimide (m.p. 92°)[2] it melted at 90–91°. Various fractions of slightly lower melting points were obtained and all of these had their melting points raised when admixed with an authentic specimen.

GUELPH, ONTARIO, CANADA

[CONTRIBUTION FROM VENEREAL DISEASE EXPERIMENTAL LABORATORY, U. S. PUBLIC HEALTH SERVICE, UNIVERSITY OF NORTH CAROLINA, SCHOOL OF PUBLIC HEALTH]

Two Dimensional Paper Chromatography of Proteins

BY HENRY TAUBER AND EDWARD L. PETIT

RECEIVED DECEMBER 10, 1951

A protein staining reagent, containing the fluorescent dye eosin, and methyl orange has been developed for locating the movement of proteins in two-dimensional filter paper chromatography. Improvements in the two-dimensional technique have been described.

Introduction

Franklin and Quastel[1] have claimed that some proteins are separable by two dimensional filter paper chromatography using buffer solutions and the ascent principle.[2] These investigators converted the proteins into protein–hemin complexes and identified the position of each protein by streaking the paper with benzidine and hydrogen peroxide. Their method, however, has limited use since some proteins do not react with hemin, and when applicable, the color formation is so intense that a background develops which obscures the color given by the protein–hemin complex. To overcome this interference the patterns must be immediately photographed.

In 1937 Feigl and Anger,[3] and more recently others[4,5] found that dyes may be used for the detection of proteins on filter paper. There is, however, no detailed procedure available concerning the applicability of this principle to two dimensional paper chromatography. We have developed a convenient staining reagent containing the fluorescent dye, eosin, and methyl orange. Using this reagent the outlines of the patterns on the paper may be checked while damp, with the aid of ultraviolet light (Mineralight). The stained chromatograms offer a permanent record. A few two dimensional protein chromatograms are presented.

Experimental

Protein Solutions.—Twenty mg. of protein is dissolved in 1 ml. of sodium chloride–phosphate buffer of *p*H 7.5.[6] Five 0.01-ml. aliquots (total weight of protein 1000 γ) are applied to the paper for chromatography unless otherwise stated. When blood serum is used, two 0.01-ml. aliquots suffice.

Staining Solution.—Citric acid (1.5 g.) is dissolved in 1263 ml. of distilled water. Glycerol (51 ml.) and 3 l. of acetone are added. After mixing, 0.6 g. of methyl orange (Eastman Kodak 432) and 0.6 g. Eosin Y (Harleco, water and acetone soluble) are dissolved.

Application and Developing.—Whatman No. 1 filter paper is cut into 26.5 × 26.5 cm. squares (23 × 23 cm. may also be used). Border lines are drawn in pencil 2.5 cm. from the edge of the paper. The protein solutions are applied with a 0.1 ml. pipet 2.5 cm. inside the lines in the lower left-hand corner. The papers are dried at 37° in a tissue drier with circulating air supply after each application. After the last drying a hole is punched in each of the upper corners of the papers and then the papers are attached with strings to glass rods in a tank.

Two aquarium tanks (52 × 31 × 26 cm.), one for the first and one for the second dimension, are employed. Each tank contains 5 l. of the specific aqueous developing solution. One-half inch of Plasticine is placed around the top of each tank. Five (eight if the smaller paper is used) glass rods, spaced evenly, are embedded in the Plasticine at each end of the tank. Each chromatogram is immersed 2.5 cm. into the developing solution. The tanks are covered with glass plates, pressed firmly into place and sealed, to maintain constant humidity within the tanks. The chromatograms are removed when the developing solution reaches the line 2.5 cm. from the top. Then the papers are dried and returned in the tanks at 90° angle. Our experiments were carried out in a constant temperature room at 24°. Each dimension requires about 90 minutes and each series of chromatograms is completed in about 5 hours.

Staining.—The staining solution is placed in an appropriate pan. In another pan is placed 8 l. of tap water, of about 45°, acidified with 0.5 ml. of concentrated sulfuric acid. After drying, the filter paper sheets are stained for 5 minutes, then transferred into the acidified water to remove excess stain. This requires about 5 minutes. Then the sheets are placed between filter paper for partial drying. Final drying is carried out at 37°. When very dilute protein solutions (less than 100 γ) are employed, the protein outlines may temporarily disappear during drying due to the darkening of the background. The outlines reappear immediately, however, if immersed in the acidified water.

Filter Paper Chromatography of a Few Proteins.—Cytochrome c (Wyeth) in a concentration of 110 γ was subjected to two dimensional filter paper chromatography. 0.1 *M* sucrose solution of *p*H 6.6[7] was the developing medium in the first dimension and 0.1 *M* sodium potassium tartrate of *p*H 7.0[7] was employed in the second dimension (Pattern A).

Pattern B shows the chromatogram given by once recrystallized, but highly active (Kat. f. value 32,000)[8] cow

(1) A. E. Franklin and J. H. Quastel, *Science*, **110**, 447 (1949).
(2) R. J. Williams and H. Kirby, *ibid.*, **107**, 481 (1948).
(3) F. Feigl and V. Anger, *Microchemica Acta*, **2**, 107 (1937).
(4) J. I. M. Jones and S. E. Michael, *Nature*, **165**, 685 (1950).
(5) S. C. Papastamatis and J. F. Wilkinson, *ibid.*, **167**, 724 (1951).
(6) G. L. Miller and R. H. Golder, *Arch. Biochem.*, **29**, 420 (1951).
(7) A. E. Franklin, J. H. Quastel and S. F. Van Straten, *Proc. Soc. Exp. Biol. & Med.*, **77**, 783 (1951).
(8) H. Tauber and E. L. Petit, *J. Biol. Chem.*, **195**, 703 (1952).