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The Insecticidal Principles of *Haplophyton cimicidum*. I. Haplophytine[1]

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A larger scale, more efficient method of extraction and isolation of the alkaloids haplophytine and cimicidine has been developed. Haplophytine has the empirical formula $C_{27}H_{31}N_3O_5$. It contains two basic nitrogen atoms (one weakly so) and at least one N-methyl group. The basic nitrogen atoms are responsible for the reaction of haplophytine with cyanogen bromide, carbon tetrachloride and sulfur dioxide. The reason for the acidic properties of the alkaloid has not been determined. The molecule contains two methoxyl groups and probably two carbonyls. Haplophytine is readily hydrolyzed under basic or acidic conditions, but the products could not be purified due to their high water solubility. Hydrogenation of haplophytine indicates the presence of a carbon-to-carbon double bond but, if present, it is unattacked by osmium tetroxide. Hydrogen peroxide and potassium permanganate react with haplophytine, but the reactions do not involve a carbon-to-carbon double bond. Alkaline hydrogen peroxide converts the alkaloid to a weakly basic compound, $C_{21}H_{24}N_2O_5$. When haplophytine is gently oxidized with potassium permanganate, a crystalline product, $C_{27}H_{29}N_3O_6$, is isolated.

Haplophytine, empirical formula $C_{27}H_{31}O_5N_3$, and cimicidine, empirical formula $C_{23}H_{28}O_5N_2$, are insecticidal alkaloids which were isolated from the Mexican plant *Haplophyton cimicidum* in yields of 0.034% and 0.003%, respectively.[4] The yields were drastically reduced in later work, apparently due to the use of more mature plant as a source of the alkaloids. This and the following communication describe an improved extraction and isolation procedure as well as some of the chemistry of the alkaloids.

Previous extraction experiments were performed using a percolation technique on about 7 kg. of ground plant[4]; the time required for the isolation was from six to seven weeks. Extraction experiments were begun using a Brighton solid–liquid extractor. The percolation vessel of this extractor held approximately 22 kg. of loosely-packed ground plant. The refluxing solvent could percolate continuously through the plant and the extract was collected in the solvent boiler. Extraction solvents employed were chloroform and methanol–chloroform (19:1). The ground plant was covered with solvent, the mixture was let stand for five days and then the solvent was continuously percolated through the plant for 24 hours. The best results were obtained with methanol–chloroform as an extraction solvent, but these extracts were also the most difficult to work up. The plant extract was fractionated with acid and base to yield crude amphoteric alkaloid. Chromatography of the crude amphoteric alkaloid yielded a mixture of haplophytine and cimicidine. This alkaloid mixture could be induced to crystallize from ethanol, but repeated recrystallization gave only partial separation of the alkaloids. The alkaloids were separated by extraction of a chloroform solution of the mixed alkaloids with dilute citric acid solution. Cimicidine was quantitatively extracted while haplophytine was extracted to only a slight extent. The yield of haplophytine by this method of extraction and isolation was approximately 0.01% and the yield of cimicidine varied from 0.002% with chloroform as the extraction solvent to 0.003% with methanol–chloroform.

Although haplophytine and cimicidine are the first alkaloids to be isolated from *H. cimicidum*, many alkaloids have been isolated from other plants of the family *Apocynaceae*.[5] Those whose structures are completely known are quebrachine (yohimbine), alstonine,[6] serpentine,[7] conessine and conkurchine.[8] The first three named are alkaloids which may be considered derived from indole, and the last two are steroidal alkaloids. A large number of those which are incompletely known also appear to belong to the indole group. The ultraviolet absorption spectra of both haplophytine and cimicidine[4] indicate the possible presence of indole or carbazole-like groups, but no chemical evidence is yet available on this point. Indeed, all the qualitative tests for indole or pyrrole groups were negative and no indole odor was noted during zinc dust distillation.

The analytical data for seven independently prepared crystalline samples of haplophytine (including one sample which had been sublimed) were all in agreement with the empirical formula $C_{27}H_{31}N_2O_5$.[4] The following amorphous salts have been prepared in analytical purity: dihydrochloride, twice; monopicrate, twice; dihydrobromide, once; the salt with two moles of acetyl chloride, once.

From the formation of diacidic salts it is clear that two of the three nitrogen atoms are basic in character. Furthermore, titration studies of the dihydrochloride gave evidence that one of the nitrogen atoms is a much stronger base than the other; considerable interference was evident at the phenolphthalein end-point and only at the thymolphthalein end-point (*p*H 10.5–11) was titration complete. Neutralization equivalents of about 260 were obtained when this indicator was used (theoretical 275); the neutralization equivalent of the dihydrobromide was 290 (theoretical 319). The somewhat low values might be due to some interfering titration of the weakly acidic group in the molecule. Formation of a monopicrate may also be taken as evidence that one amine function is more basic than the other.

The basic nitrogen atoms of haplophytine are also responsible for the reaction of the alkaloid with cy-

(1) Grateful acknowledgement is made of the partial support of this research by a grant from the National Science Foundation (G135).

(2) E. I. du Pont de Nemours and Company Fellow, 1950–1951.

(3) Procter and Gamble Company Fellow, 1952–1953.

(4) E. F. Rogers, H. R. Snyder and R. F. Fischer, THIS JOURNAL, **74**, 1987 (1952).

(5) R. Seka in G. Klein, "Handbuch der Pflanzenanalyse," Vol. IV, Julius Springer, Wien, 1933, p. 735; T. H. Henry, "The Plant Alkaloids," The Blakiston Company, Philadelphia, Penna., 1949.

(6) R. C. Elderfield and A. P. Gray, *J. Org. Chem.*, **16**, 506 (1951).

(7) E. Schlittler and H. Schwarz, *Helv. Chim. Acta*, **33**, 1463 (1950).

(8) A. Bertho, *Ann.*, **569**, 1 (1950).

anogen bromide, carbon tetrachloride and sulfur dioxide. When haplophytine was treated with cyanogen bromide, a basic and a neutral product were isolated. The basic product predominated after a reaction time of one day and the neutral product after four days. The infrared spectra of the two products were similar, and differed mainly in aliphatic C–H absorption and in the strength of the CN band at 2225 cm.$^{-1}$. It would appear that an N-alkyl group was attacked in both cases, but further work is needed before any conclusions can be drawn. Haplophytine reacted with carbon tetrachloride on a mole-for-mole basis to yield an ill-defined, water-soluble, salt-like product. When haplophytine was dissolved in liquid sulfur dioxide, removal of the sulfur dioxide yielded a bright yellow solid which had the composition of haplophytine plus one molecule of sulfur dioxide. The infrared spectrum of the compound was almost identical to that of haplophytine. If sulfur dioxide was passed through a chloroform solution of haplophytine a bright yellow color developed, but removal of the chloroform yielded only colorless haplophytine. Relatively unstable, colored compounds formed by the addition of sulfur dioxide to a basic nitrogen atom are quite well known[9] and the above compound is undoubtedly of this type.

There is considerable evidence that all three nitrogen atoms of haplophytine are tertiary. The infrared absorption spectrum (4) displays no significant absorption in the region 3700–3000 cm.$^{-1}$ and the only reaction with acetyl chloride was the formation of a salt. Qualitative reactions with the very sensitive Duke reagent were also negative. The alkaloid reacted with acetic, butyric and benzoic anhydrides, but the reaction was unusual and did not seem a simple acylation. Analytical data showed that at least one N-methyl group is present and this evidence was supplemented by the isolation of methylamine (as the picrate) in the zinc dust distillates of the compound. All attempts to obtain a methiodide have been unsuccessful; in view of the formation of the acid salts, it seems possible that the formation of the methiodide is readily reversible.

The reason for the acidity of haplophytine is not yet understood. It is not a clear-cut case of salt formation because chloroform readily removes haplophytine from 0.2 *N* sodium hydroxide, while 1 *N* alkali extracts the alkaloid from chloroform. Evaporation of barium hydroxide or ammonia solutions of haplophytine yielded only haplophytine itself.

Two of the oxygen atoms of haplophytine are present as methoxyl groups and at least two are present as carbonyl groups.[4] The methylenedioxy group is absent and there is no direct evidence for the presence of a hydroxyl group although some of the reactions of the alkaloid seem to point to its possible presence.

Haplophytine can be completely destroyed by hydrolysis with hydrochloric acid or sodium hydroxide. Hydrolysis of haplophytine with barium hydroxide yielded a red, water-soluble organic product which could not be purified. It sublimed at 260° (0.5 mm.) (bath temp.), but the sublimate was badly decomposed and had a strong amine-like odor. The hydrolysis product could not be extracted from its aqueous solution by chloroform or ether at a variety of *p*H values. The infrared absorption spectrum contained a broad band at 1579 cm.$^{-1}$, probably due to an ionized carboxyl group. That the compound was not a metallic salt was shown by the fact that it burned completely and that passage of its aqueous solution through a cation exchange column (Amberlite IR-120) had no effect on the infrared spectrum. Sulfurous acid also slowly hydrolyzed haplophytine to a water-soluble product, but again its high water solubility prevented purification or characterization of the material.

When haplophytine was hydrogenated over platinum at room temperature and atmospheric pressure one mole of hydrogen was quickly absorbed and dihydrohaplophytine was isolated. The reduction product had similar properties to haplophytine except that it was less stable and it formed only monoacidic salts. The infrared spectrum of the reduction product was not remarkably different from that of the parent alkaloid (carbonyl groups unaffected). The above evidence may indicate the presence of a readily reduced carbon-to-carbon double bond in haplophytine. However, osmium tetroxide did not react with haplophytine and the reactions of alkaline hydrogen peroxide and potassium permanganate with haplophytine were not the reactions of a carbon-to-carbon double bond.

When an alkaline solution of haplophytine was treated with hydrogen peroxide a reaction took place and a crystalline compound of formula $C_{21}H_{24}O_5N_2$, corresponding to the loss of C_6H_7N, was isolated. The product is weakly basic and still contains two methoxyl groups, the N-methyl group and the carbonyl groups which absorb at 1751 cm.$^{-1}$ and 1656 cm.$^{-1}$ in the infrared. The compound is sensitive to further oxidation with peroxide, but no crystalline product was isolated from the extended oxidation.

Haplophytine is very readily attacked by dilute aqueous permanganate solution. When the oxidation was stopped after the addition of permanganate equivalent to two atoms of oxygen per mole of haplophytine, a crystalline compound was isolated. This oxidation product has almost no basic properties, but its acidity is considerably increased. It was not attacked by alkaline hydrogen peroxide. The infrared spectrum of a chloroform solution of the oxidation product showed absorption peaks at 1745, 1725, 1655 and 1600 cm.$^{-1}$. If the infrared spectrum was determined on a Nujol mull of the oxidation product, the absorption peaks were at 1747, 1734, 1642 and 1588 cm.$^{-1}$ and, in addition, there was some absorption around 3150 cm.$^{-1}$, possibly due to O–H stretching vibration, and at 1150 cm.$^{-1}$, possibly due to C–O stretching vibration. The reason for these rather large differences in the two spectra is not known. Analysis of the oxidation product showed the loss of two hydrogen atoms and the gain of one oxygen atom during the course of the oxidation. This result corresponds to the oxidation of a primary alcohol group to a car-

(9) L. C. Bateman, E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 243 (1944).

boxylic acid, oxidation of a methylene group to a ketone group or oxidation of a lactone to a dicarboxylic acid which spontaneously forms an anhydride. No indication of a primary alcohol group has been detected in haplophytine, but the Nujol mull spectrum of the permanganate oxidation product can be correlated with a carboxylic acid. The oxidation product reacted with diazomethane to give an extremely small amount of an amorphous solid which, from its infrared spectrum, appeared to be an ester but, since the "ester" was isolated in such small yield and was not pure, a definite conclusion cannot be drawn. The second possibility for the permanganate oxidation of haplophytine, the production of a ketone group from a methylene group, has not been proven or disproven although this type of reaction is unusual for potassium permanganate and cannot readily explain the acidity of the product. The production of an acid anhydride can account for the acidity of the oxidation product. However, haplophytine does not react with hydrazine hydrate and the oxidation product does not react with aniline so that considerable doubt is cast on the existence of a lactone in haplophytine or an anhydride in the oxidation product. The infrared spectrum of an acid anhydride would be expected to show two absorption bands about 50 cm.$^{-1}$ apart and at slightly lower wave lengths than the absorption peaks exhibited by the oxidation product. A large ring anhydride might show absorption near the observed frequencies but spontaneous formation and reversible opening of a large ring anhydride are extremely unlikely. The determination of the nature of the permanganate oxidation product will thus have to await further work.

Experimental[10]

Extraction and Isolation of Haplophytine and Cimicidine.—Fifty pounds (22 kg.) of ground (1/2" screen), authentic *Haplophyton cimicidum* was placed in the percolation unit of the solid–liquid extractor, 200 lb. of solvent was added and the mixture was let stand. After five days, 50 lb. of solvent was added to the solvent boiler and the reflux was allowed to percolate through the plant for 24 hours. The solvent was then concentrated to about 6 l. and removed from the solvent boiler.

The solvents used were chloroform and methanol–chloroform (19:1). The chloroform extracts were filtered through Filter-cel before further work-up. When methanol–chloroform was used as the extraction solvent, the extract taken from the solvent boiler was concentrated, under reduced pressure, to about 1 l. The thick dark mass was treated with 5 l. of chloroform and the chloroform was siphoned from the insoluble material. The insoluble material was treated with a further 1-l. portion of hot chloroform and the chloroform solution was again siphoned from the insoluble material. The combined chloroform solutions were used for the isolation procedure.

The chloroform solutions of the plant extracts obtained from the Brighton solid–liquid extractor were worked up in approximately 2-l. portions. The chloroform solution was treated four times with 200-ml. portions of 2 *N* hydrochloric acid solution. The acid layers were separated, cooled with crushed ice and then brought to *p*H 8 with a saturated sodium carbonate solution. The aqueous suspension was extracted with four 200-ml. portions of chloroform and the combined chloroform layers were again extracted with four 200-ml. portions of 2 *N* hydrochloric acid solution. The acid layers were cooled with crushed ice, brought to *p*H 8 as before and extracted with four 200-ml. portions of chloroform. The combined chloroform layers were extracted with four 125-ml. portions of 5% sodium hydroxide solution. The alkaline extracts were cooled with crushed ice and brought to *p*H 8 with concentrated hydrochloric acid. The resulting aqueous suspension was extracted with three 100-ml. portions of chloroform; the combined chloroform layers were washed once with water and then taken to dryness on the steam-bath under reduced pressure to yield the dark brown, easily powdered, crude amphoteric alkaloid. From a typical extraction of 22 kg. of ground plant, 12 g. of crude amphoteric alkaloid was obtained.

The crude amphoteric alkaloid was dissolved in the minimum quantity of pure, dry, alcohol-free chloroform and introduced onto a column of alumina (Merck, suitable for chromatographic adsorption). The quantity of alumina used was varied, but the best results were obtained when the weight of alumina equalled five times the weight of crude alkaloid. After introduction of the crude alkaloid, 2 l. of chloroform was passed through the column. The chloroform was removed from the eluate, in one case, and replaced by a few ml. of absolute ethanol. A white crystalline solid separated from the solution after about 12 hours. From 3.6 g. of crude alkaloid, 0.750 g. of white, crystalline solid was obtained, m.p. 255–265° dec. Recrystallization of this mixed alkaloid from chloroform–ethanol had only a slight effect on the melting point. In some cases, more mixed alkaloid was obtained by elution of the alumina column with 1-l. portions of chloroform–acetone (9:1) and chloroform–methanol (9:1). The alkaloid obtained from these latter eluates was rather impure and could not always be induced to crystallize.

A portion of the mixed alkaloid (0.500 g.) was dissolved in 100 ml. of chloroform and extracted with four 10-ml. portions of 2 *N* citric acid solution. The chloroform layer was then taken to dryness on the steam-bath under reduced pressure and the white residue was recrystallized from chloroform–ethanol to give 0.400 g. of pure haplophytine, m.p. 293–296° dec. (dark from 275°).[11] The combined citric acid layers were brought to *p*H 8 with 5% sodium hydroxide solution and the aqueous solution was extracted four times with 25-ml. portions of chloroform. The chloroform was removed from the combined chloroform layers on the steam-bath under reduced pressure and the white residue was crystallized from chloroform–ethanol to yield colorless prisms, m.p. 266–269° dec. (dark from 245°).[11] One recrystallization from acetone–ethanol gave pure, highly crystalline cimicidine, m.p. 268–270° dec. (dark from 250°).[11,12]

In subsequent isolations the mixed alkaloid was not isolated. The chloroform eluted from the chromatography of the crude amphoteric alkaloid was concentrated to 100 ml. and treated with 2 *N* citric acid solution as above. The yield of haplophytine, based on the weight of ground plant extracted, by this method of extraction and isolation was approximately 0.01%. The yield of cimicidine varied from 0.002%, when chloroform was used as the extraction solvent, to 0.003% when methanol–chloroform was used as the extraction solvent.

Color, Precipitation and Qualitative Reactions.—Haplophytine dissolved in concentrated sulfuric acid, forming a yellow solution, which gradually darkened to orange-red on standing. With concentrated nitric acid, it gave a very deep blue color, which gradually faded; when the solution was evaporated to dryness and moistened with alcoholic alkali (Vitali's test for esters of the tropic acid series),[13] no color was obtained. The alkaloid dissolved in 5% ferric chloride solution, but no color change occurred until the solution was evaporated to dryness. The following color reactions for pyrroles and indoles were negative: the pine-splint test,[14] the Adamkiewicz test[15] (concd. sulfuric acid

(10) Infrared spectra were determined and interpreted by Mrs. H. P. Leighly and Miss Helen Miklas using a Perkin–Elmer double beam spectrophotometer. Analyses were performed by Mr. Joseph Nemeth, Mrs. Lucy Chang, Mrs. Esther Fett and Mrs. Katherine Pih.

(11) Melting point determined on a calibrated, Hershberg type melting point apparatus using open capillaries; the initial temperature was room temperature.

(12) Reported melting point of cimicidine is 259–262°.[4] The presently isolated cimicidine is undoubtedly purer than that previously isolated. The analyses and infrared spectra are identical. The homogeneity of the alkaloid was established by chromatography.

(13) G. Trier and E. Winterstein, "Die Alkaloide," Gebrüder Borntraeger, Berlin, 1931, p. 968.

(14) A. A. Morton, "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1946, p. 68.

(15) *Ibid.*, p. 350.

and ferric chloride), all variations of the Ehrlich reaction[16] (*p*-dimethylaminobenzaldehyde), the Hopkins–Cole test[17] (sulfuric acid and glyoxylic acid) and del Guerra's reaction[18] (dichromate and hydrochloric acid). The Vongerichten test[19] for pyridines (2,4-dinitrochlorobenzene and alkali) was inconclusive, because haplophytine itself gives a brilliant red-purple color when warmed with alcoholic alkali.

Amorphous precipitates were obtained (from acid solution) with Reinecke salt, phosphomolybdic acid, chloroplatinic acid, and mercuric chloride. All of these products decomposed during attempted crystallization from water or alcohol; in one case, the mercuric chloride complex was decomposed with 5% alkali, the *p*H was adjusted to 8, and the solution was extracted with chloroform. Upon replacement of this solvent by ethanol, pure haplophytine crystallized. No precipitate was obtained (from acid or ammoniacal solution) with silver nitrate, cupric chloride or oxalic acid.

The following qualitative tests were also negative: sulfuric acid–phloroglucinol test for methylenedioxy,[20] 2,4-dinitrophenylhydrazine and Benedict tests for aldehyde or ketone,[21] iodoform reaction, periodic acid test and Duke's tests for primary and secondary amine groupings. Qualitative elemental analysis confirmed the presence of nitrogen and the absence of sulfur and halogen.

Haplophytine is soluble in concentrated ammonia solution and in saturated barium hydroxide, but evaporation of the solutions, in the air or *in vacuo*, resulted only in the reprecipitation of the original alkaloid.

Haplophytine Hydrochloride.—A solution of 15–20 mg. of pure alkaloid (dec. 288–292°) was prepared in 2 ml. of dry chloroform and 5–6 ml. of dry ether. Dry hydrogen chloride was passed through the solution until precipitation was complete, and the pale-yellow flocculent precipitate was separated by centrifugation. After two washings with dry ether, the precipitate was allowed to dry in a desiccator and dried to constant weight *in vacuo*. In one experiment, 14.5 mg. of alkaloid gave 16.9 mg. of hydrochloride (corresponds to $C_{27}H_{31}O_5N_3 \cdot 2HCl$), m.p. 208–218° dec. (darkening from 200°).[22] The product was stable in the dry state, but was partially decomposed when dissolved in water, alcohols or acetone. Titrations of aqueous solutions gave neutralization equivalents of 337 ± 10 (phenolphthalein end-point) or 260 ± 20 (thymolphthalein end-point): theoretical, 275. Haplophytine was recovered in about 90% yield by treatment of the aqueous solution with sodium carbonate (to *p*H 8), extraction with chloroform and crystallization from ethanol. For analytical purposes, the above preparative procedure was followed by washing with carefully purified and dried solvents.

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot 2HCl$: C, 58.91; H, 6.04; N, 7.63; Cl, 12.88. Found: C, 59.20, 59.02;[23] H, 6.05, 6.06; N, 7.46; Cl, 13.56.

Picrate.—To a solution of 40–50 mg. of haplophytine in a mixture of 25% dry chloroform and 75% dry ether, saturated picric acid in ether was added dropwise until precipitation was complete. The bright-yellow suspension was centrifuged, washed once with dry ether, and then redissolved in a minimum quantity of chloroform. The solution was centrifuged to remove extraneous materials, and the clear solution was added dropwise to 20 volumes of dry ether in a test-tube. After centrifugation and two washings with small quantities of dry ether, the bright-yellow, powdery product, m.p. 140–160° dec.,[22] was dried at 100° for five hours.

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot C_6H_3O_7N_3$: C, 56.08; H, 4.85; N, 11.89. Found: C, 55.90, 56.22; H, 4.74, 4.88; N, 11.71.

The picrate was very soluble in water, alcohols, acetone and chloroform, but insoluble in ether and petroleum ether. Attempts to crystallize the compound were unsuccessful; from ethanol–cyclohexane, a brownish powder could be obtained, m.p. 163–164° (instantaneous melting when dropped on the hot-stage).

Anal. Found: C, 57.65, 57.43; H, 5.18, 5.27; N, 10.92.

Hydrobromide.—One drop (*ca.* 0.05 ml.) of 48% hydrobromic acid was added to 20–30 mg. of pure haplophytine dissolved in 2–3 ml. of acetone. A white precipitate formed, which was treated with 6–8 ml. of ether immediately, centrifuged and washed with dry ether to give a white powder, m.p. 200–203° dec.[22] This material, however, slowly darkened even in the dry state; it could be obtained in a reasonably stable form by taking up the freshly-formed, dry hydrobromide in pure methanol and precipitating it into ether. Centrifugation, followed by two careful washings with dry ether gave a very pale yellow, hygroscopic powder, decomposing at 200–202°.[22] Its properties were very similar to those of the hydrochloride.

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot 2HBr$: C, 50.72; H, 5.20; N, 6.57; neut. equiv., 319. Found: C, 51.03; H, 5.54; N, 6.15; neut. equiv., 290 ± 20 (thymolphthalein).

Acetyl Chloride Salt.—A solution of 15–20 mg. of pure haplophytine was prepared in 6–7 ml. of a mixture of 1/3 dry benzene and 2/3 dry ether. Pure acetyl chloride was added dropwise until precipitation was complete. The pale-yellow powder was removed by centrifugation and washed twice with dry ether; it was dried *in vacuo* first in a desiccator and then at 100° over phosphoric anhydride for four hours. The originally almost colorless powder darkened somewhat at 100°, but this drying temperature was necessary to effect removal of the last traces of water. The salt darkened rapidly at 200°, shrank from 205–215°, and blackened from 215–230°.[22]

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot 2CH_3COCl$: C, 58.67; H, 5.88; N, 6.62. Found: C, 58.46; H, 5.77; N, 6.65.

This salt was soluble in water, alcohols or acetone, with some decomposition. Addition of aqueous sodium carbonate resulted in evolution of carbon dioxide, and extraction of the resulting solution at *p*H 8 with chloroform, followed by crystallization from ethanol, gave a few milligrams of crystalline product having an infrared absorption spectrum identical with that of haplophytine. A benzoyl chloride salt was formed similarly, but was not further investigated.

Reaction of Cyanogen Bromide with Haplophytine.[24]—To a solution of 100 mg. of cyanogen bromide[25] in 5 ml. of chloroform was added a solution of 150 mg. of haplophytine in 5 ml. of chloroform. The solution was let stand overnight; a slight orange color developed. The reaction mixture was extracted five times with 5-ml. portions of 2 *N* hydrochloric acid solution. The chloroform was removed from the extracted chloroform solution; the residue was a trace of brown oil. The combined acid layers were neutralized with sodium carbonate and extracted five times with 5-ml. portions of chloroform. The chloroform was removed from the combined chloroform layers to leave 45 mg. of a brown solid. The solid material was dissolved in chloroform and chromatographed on 0.6 g. of alumina (Merck, suitable for chromatographic adsorption). The first 15 ml. of eluate (chloroform) yielded 15 mg. of a white solid. The solid was crystallized from ether–alcohol, m.p. 283–287° dec. (dark from 250°).[11] The material had a similar absorption spectrum to haplophytine except for a small peak at 2225 cm.$^{-1}$ (which could be due to the presence of the C≡N group).

The reaction was repeated and the reaction mixture was let stand in the dark for four days. The reaction mixture was worked up as above; there was only a trace of basic product present. The neutral product was isolated as a brown solid (approximately 50 mg.). The neutral product was recrystallized from ether–alcohol to yield an off-white powder. The infrared spectrum of this neutral, cyanogen

(16) (a) H. Fischer and H. Orth, "Die Chemie des Pyrrols," Vol. I. Akademische Verlagsgesellschaft m.b.H., Leipzig, 1934, p. 66; (b) E. Salkowski, *Biochem. Z.*, **97**, 123 (1919); (c) H. W. van Urk, *Pharm. Weekblad*, **66**, 101 (1929); *C. A.*, **23**, 1717 (1929); (d) A. H. Cook and J. R. Majer, *J. Chem. Soc.*, 488 (1944).

(17) P. B. Hawk, B. L. Oser and W. H. Summerson, "Practical Physiological Chemistry," The Blakiston Co., Philadelphia, Pa., 1947, p. 155.

(18) G. del Guerra, *Arch. Farmacol. Sper.*, **59**, 86 (1935); *C. A.*, **29**, 2886 (1935).

(19) S. P. Vilter, T. D. Spies and A. P. Mathews, *J. Biol. Chem.*, **125**, 85 (1938).

(20) G. O. Gaebel, *Arch. Pharm.*, **248**, 226 (1910).

(21) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948.

(22) Melting point determined on a calibrated Fisher block.

(23) Where more than one analysis is reported, the work was done on independently prepared samples.

(24) H. A. Hagemann, "Organic Reactions," Vol. VII, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 198.

(25) W. W. Hartmann and E. E. Dreger, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 150.

bromide product was similar to that of the basic product; it differed mainly in that the absorption at 2225 cm.$^{-1}$ was stronger and the C–H absorption was altered.

Reaction of Haplophytine with Sulfur Dioxide.—When sulfur dioxide was passed through a solution of 25 mg. of haplophytine in 5 ml. of chloroform, the solution immediately developed a bright yellow color. The chloroform was removed by distillation and replaced by ethanol. A white, crystalline material slowly separated from the alcohol solution and was collected; m.p. 285–290° dec. (dark from 275°).[11] The infrared spectrum of the material was identical to that of haplophytine.

When 33.8 mg. of analytically pure haplophytine was dissolved in 2 ml. of pure, liquid sulfur dioxide, the characteristic bright yellow color developed immediately. The sulfur dioxide was removed at room temperature under reduced pressure to yield 38.7 mg. of a bright yellow, glassy solid. The gain in weight was 4.9 mg. (theoretical gain in weight if one mole of sulfur dioxide added to one mole of haplophytine is 3.4 mg.). The infrared spectrum of the yellow solid showed no observable differences from that of haplophytine. When the yellow solid was heated, a definite change was noted at 195°; the material melted with decomposition at 274–280°.[11] The material was dried at 61° for seven hours and at 20° for 19 hours *in vacuo* over phosphoric anhydride prior to analysis.

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot SO_2$: C, 59.87; H, 5.77. Found: C, 61.17; H, 5.91.

Reaction with Carbon Tetrachloride.—Haplophytine was slowly soluble in carbon tetrachloride; after about an hour, the solution became cloudy, and within a few hours an amorphous tan precipitate rose to the surface. After the reaction was complete (24 hours) ether was added, and the product was removed by centrifugation; 0.1008 g. of alkaloid gave 0.1359 g. of product (corresponds to one mole of carbon tetrachloride per mole of alkaloid).

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot CCl_4$: C, 53.26; H, 4.95. Found: C, 53.91, 53.93; H, 5.11, 5.29.

This product was soluble in alcohols and water, and the aqueous solution gave an immediate, copious precipitate with aqueous silver nitrate. Extraction of the aqueous solution (*p*H 8) with chloroform and evaporation of the solvent gave a brown resin which could not be induced to crystallize. The reaction with carbon tetrachloride took place readily in the presence of chloroform, but no precipitation occurred with chloroform solutions of carbon tetrabromide, hexachloroethane or benzotrichloride. A similar reaction did occur, however, with benzotrichloride alone, and with pentachloroethane.

Zinc Dust Distillations.—The apparatus and procedures described by Witkop[26] were employed, with certain necessary modifications. The distillation tube was heated electrically, and the circuit was adjusted so that a temperature of 370–380° was reached in two minutes; this temperature was maintained for ten minutes. At higher temperatures or longer periods of heating, the distillate was highly colored, and at lower temperatures only minute amounts were obtained. A fresh bottle of best quality zinc was used, and the nitrogen passing through the system was freed of oxygen and water. The distillation mixture consisted of 15 parts of zinc dust mixed intimately with one part of haplophytine. Each distillation was performed on 0.6 g. of this mixture, and each charge was preceded by 0.1 g. of pure zinc dust. "Pyrex wool" was superior to asbestos for use as a plug at the constriction of the combustion tube. It was found convenient to have several such tubes on hand, and the insertion of a fine copper wire before the distillation greatly facilitated removal of the residues.

The colorless or pale-yellow distillates were condensed in a Dry Ice trap; each distillation afforded at most 0.005 ml. of an oil with a fishy (occasionally quinoline-like) odor. No trace of an indole odor was detectable. When eleven such distillates were combined in ether, they darkened very rapidly. During the fractionation into alkaline, neutral and acidic fractions, further darkening occurred, and all three fractions rapidly polymerized to black, insoluble tars. Nothing crystalline could be isolated from these tars, and an attempted chromatographic separation of the alkaline fraction was fruitless.

(26) B. Witkop, *Ann.*, **554**, 83 (1943); H. Wieland, B. Witkop and K. Bähr, *ibid.*, **558**, 144 (1947).

Some success was attained by adding the ethereal picric acid directly to the distillate in the condenser immediately after distillation; under these conditions truly crystalline picrates could be obtained. They were washed quickly with ether, and dissolved in methanol. This solvent was then replaced by benzene at room temperature *in vacuo*, and the picrate (or picrates) crystallized in very fine orange needles, which darkened more or less rapidly. From 13 combined distillates, a total of 14–16 mg. of crystalline product was obtained; m.p. 180–183° dec. This was recrystallized once from benzene; m.p. 183–186° dec.[22] The analysis indicated that the product was slightly impure methylamine picrate.

Anal. Calcd. for $CH_3NH_2 \cdot C_6H_3O_7N_3$: C, 32.32; H, 3.10; N, 21.54. Found: C, 32.88; H, 3.49; N, 21.01.

A comparison of the infrared absorption spectra of this picrate and of authentic methylamine picrate showed them to be identical. A further small-scale recrystallization from ethyl acetate gave needles, m.p. 186–189° dec.,[22] (lit. 207°).[27]

Attempted Formation of Methiodide of Haplophytine.—Unchanged starting material was recovered after treatment of haplophytine in benzene or chloroform with methyl iodide at room temperature, at reflux in the above solvents, or from periods of reflux in pure methyl iodide as long as 21 hours.

Unchanged haplophytine was recovered also after a brief period of reflux with ethyl bromide; with benzyl chloride and pentamethylene dibromide, however, reactions occurred, since the solutions turned red and then brown, slowly at room temperature, and rapidly if warmed. Nothing crystalline could be isolated from these reaction mixtures.

Hydrogenation of Haplophytine.—At room temperature and pressure, 1.07 moles of hydrogen was absorbed per mole of alkaloid (mean of three runs) within 20 minutes, with methanol as the solvent and prereduced platinum oxide as the catalyst; no further hydrogen uptake was noted after several hours. In a typical case, 0.1055 g. of haplophytine was hydrogenated in 10 ml. of methanol over 0.0286 g. of prereduced platinum oxide at 27° and 747 mm.; the hydrogen uptake was 5.4 ml. (0.97 mole per mole). The specific rotation of the solution at the completion of reduction was +144° (1%, methanol). A similar uptake (0.84 mole per mole) was obtained when the reduction was conducted in 0.1 *N* sodium hydroxide (platinum oxide catalyst), and the product obtained was identical with that obtained from the hydrogenation in methanol. However, when prereduced 10% palladous chloride on charcoal was employed as catalyst, 3.7 moles of hydrogen was absorbed per mole of haplophytine, and the resulting solution was greenish-yellow and had no optical activity. The solution darkened rapidly and only a black resin could be obtained. With 10% palladium-on-charcoal as catalyst, 0.86 mole of hydrogen per mole of alkaloid was absorbed, the resulting solution was also green, $[\alpha]^{25}D$ +47° (1%, methanol), and only resinous products could be isolated.

Dihydrohaplophytine could be isolated by replacement of the methanol by a suitable solvent, but the compound was rather unstable. Absorption maxima in the infrared region were at 1751, 1700, 1655 and 1597 cm.$^{-1}$. In one case, two crystallizations from ethyl acetate gave a material, m.p. 190–193°, $[\alpha]^{26}D$ +152° (1%, chloroform), but an attempted third crystallization resulted in the formation of a yellow resin. A freshly-prepared, amorphous product, obtained by addition of a saturated ether solution to a large volume of petroleum ether (b.p. 90–110°), gave the most satisfactory analysis.

Anal. Calcd. for $C_{27}H_{33}O_5N_3$: C, 67.62; H, 6.94; N, 8.76. Found: C, 67.66; H, 7.21; N, 8.70.

A sample, m.p. 195–198°, crystallized quickly from acetone and dried over phosphoric anhydride for five hours gave the following analysis: C, 67.20; H, 7.12. Part of this sample was used to form a picrate, which was prepared and purified by a method similar to that employed for the picrate of haplophytine. The yellow powder darkened at 150–180° and blackened rapidly at 180–190°[22]; for analysis it was dried *in vacuo* for eight hours at 100°.

Anal. Calcd. for $C_{27}H_{33}O_5N_3 \cdot C_6H_3O_7N_3$: C, 55.93; H, 5.12; N, 11.86. Found: C, 55.62; H, 5.21; N, 12.21.

During several crystallizations from acetone–water, the

(27) S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1946, p. 206.

dihydro product turned pale yellow in color and reached a constant melting point of 198–200° (change in appearance at 160°),[22] $[\alpha]^{25}D$ +160° (1%, chloroform).

Anal. Calcd. for $C_{27}H_{33}O_6N_3$: C, 65.44; H, 6.71. Found: C, 65.70; H, 6.62.

Amorphous hydrochlorides were prepared for analysis by a method similar to that employed for haplophytine hydrochloride.

Anal. Calcd. for $C_{27}H_{33}O_6N_3 \cdot HCl$: C, 60.95; H, 6.44; N, 7.90. Found: C, 61.10, 61.61; H, 6.83, 6.62; N, 8.05.

The dihydro product continued to be oxidized in contact with acetone or water; the melting point became less sharp, and eventually complete resinification occurred.

Attempted Reaction of Haplophytine with Osmium Tetroxide.—To a solution of 270 mg. of haplophytine in 5 ml. of dioxane was added a solution of 135 mg. of osmium tetroxide in 5 ml. of dioxane. The solution was let stand at room temperature for two days; a brown-violet precipitate separated from the solution. The reaction mixture was treated with a solution of 3 g. of sodium bisulfite in 40 ml. of water and the resulting solution was heated for two hours on the steam-bath. The solvents were then removed on the steam-bath under reduced pressure to leave a dark residue. The residue was extracted with absolute ethanol, the mixture was filtered and the alcoholic filtrate was taken to dryness on the steam-bath under reduced pressure. This process was repeated twice more; the final residue was 215 mg. of a white, amorphous powder, m.p. 212–215° dec.[11] The infrared spectrum indicated that it was impure haplophytine. The amorphous powder was recrystallized from chloroform–ethanol to give colorless crystals of haplophytine, m.p. 293–295° dec.,[11] identical infrared spectra. The recovery of pure haplophytine was 180 mg. (70%).

The reaction was repeated and the reaction mixture was let stand seven days. The only substance isolated was unchanged haplophytine, m.p. 293–295° dec.[11] (identical infrared spectra). The recovery was again 70%. The reaction was repeated once more with a reaction time of 14 days. Haplophytine was again recovered.

Alkaline Hydrogen Peroxide Reaction.—Two hundred milligrams of haplophytine was dissolved in 10 ml. of 1% sodium hydroxide solution. It was sometimes necessary to filter the solution to remove a small amount of brown, insoluble resin. A 10-ml. portion of commercial 30% hydrogen peroxide was added; the solution immediately became yellow, and gas evolution followed. Within an hour, a small amount of insoluble material began to separate, and the suspension was filtered after one, two, four and seven hours, through sintered glass. Usually, a further precipitate settled out overnight; this was also collected by filtration and added to the previous precipitates. The combined yields averaged 90 mg. (45% of weight of alkaloid used). Minor variations in alkali or peroxide concentrations or in filtration intervals did not materially affect the yield, although the reaction was slower with more dilute peroxide. Only darkening of the solution was noted when haplophytine was allowed to stand 48 hours in 1% sodium hydroxide solution alone.

In several runs, only one filtration—after 12 hours—was employed, but the yields were lower and erratic (15–40%). This was probably due to the fact that the insoluble product redissolved completely if allowed to remain in contact with the reagent for 36–48 hours. Evidently a further reaction occurred, since only resinous products were obtained by chloroform extraction of the clear solution at several degrees of acidity.

The crude hydrogen peroxide product is a pale yellow powder, still soluble in dilute alkali, but only slowly soluble in 1 *N* hydrochloric acid. It is soluble in chloroform, benzene, and acetone, insoluble in ether and may be crystallized from alcohol. The compound evidently exists in two forms; one has m.p. 216–219° (slight dec.), and the other decomposes at 250–260° (some darkening from 225°).[22] The two forms have identical infrared absorption spectra and are otherwise chemically and physically identical; the low-melting form is obtained in about two-thirds of the crystallizations. Two recrystallizations give pure, colorless prisms, m.p. 216–219°[22] (or 250–260° dec.), $[\alpha]^{25}D$ +112° (1–2% chloroform). Absorption peaks in the infrared region are at 1774, 1751, 1712, 1655 and 1598 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.28; N, 7.29; CH_3O—, 16.16; CH_3—N=, 3.91. Found: C, 65.77, 65.70; H, 6.02, 6.56; N, 7.50, 7.26; CH_3O—, 13.52; CH_3—N=, 3.02.

The compound is fairly stable at room temperature in acid or basic solution, but when warmed, considerable darkening occurs. When an acidic solution is poured into water, unchanged "peroxide-product" slowly crystallizes, m.p. 245–260° dec. The hydrochloride can be prepared in a manner similar to that employed in the formation of haplophytine hydrochloride. However, hydrogen chloride is slowly lost over a period of time, and the salt reverts to the original compound. When ethereal picric acid is added to an ether–chloroform solution of this compound, no precipitate forms. No reaction occurs with methyl iodide in refluxing benzene. The compound is insoluble in carbon tetrachloride, and no reaction occurs in a chloroform–carbon tetrachloride mixture. Approximately 50% of the material was recovered after prolonged treatment with methanolic diazomethane. An attempted hydrogenation was inconclusive; apparently only 0.3 mole of hydrogen was absorbed per mole of compound, and the products rapidly resinified.

Oxidation of Haplophytine with Potassium Permanganate.—Haplophytine was dissolved in 10 ml. of 0.1 *N* sodium hydroxide solution in a 3-necked flask fitted with a stirrer and a dropping funnel. The exact amount of potassium permanganate to provide two oxygen atoms per mole of haplophytine was weighed out and dissolved in 40 ml. of distilled water. In a typical experiment, 90 mg. of haplophytine was oxidized with 40 mg. of potassium permanganate. The permanganate solution was added dropwise to the haplophytine solution over the course of 30 minutes with stirring of the reaction mixture. The stirrer and dropping funnel were then removed from the reaction flask and sulfur dioxide was passed through the solution until the *p*H was just below 3; the manganese dioxide dissolved and a flocculent white precipitate formed. The acidified reaction mixture was extracted ten times with 5-ml. portions of ether, the ether was dried over sodium sulfate and then removed with an air stream to yield an almost white residue. From 90 mg. of haplophytine, 45 mg. of oxidation product was obtained. The product after one recrystallization from ethanol consisted of pure white crystals, m.p. 304–306° dec. (dark from 280°),[11] $[\alpha]^{25}D$ +209°. The infrared spectrum of the oxidation product had absorption peaks at 1745, 1725, 1655 and 1600 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{29}O_6N_3$: C, 65.97; H, 5.95; N, 8.55. Found (dried 100°, 4 hr.): C, 65.88; H, 6.21; N, 8.36.

This oxidation product was insoluble in water, but readily soluble in even very dilute alkali; it dissolved in 5% sodium bicarbonate solution, provided a trace of sodium carbonate was present, but gas was not evolved. The compound dissolved very easily in aqueous ammonia, but evaporation of the solution afforded only the oxidation product itself. It was much less soluble in acid than any other compound yet encountered in the haplophytine series. It dissolved incompletely in 2 *N* hydrochloric acid, and only slowly in 6 *N* acid. It also dissolved in 72% perchloric acid solution, but dilution and partial neutralization caused precipitation of the unchanged compound, m.p. 304–306° dec.[11] No precipitate was formed when ethereal picric acid was added to a chloroform–ether solution; however, when dry hydrogen chloride was passed through such a solution, a flocculent precipitate settled out; m.p. 230–240° dec. On treatment with water, this product evidently lost hydrogen chloride, since the decomposition point rose, and eventually only an inconclusive test was given with aqueous silver nitrate. The oxidation product did not react with carbon tetrachloride in the presence of chloroform or with 2,4-dinitrophenylhydrazine.

Attempted Reaction of the Permanganate Oxidation Product with Aniline.[28]—A solution of 40 mg. of permanganate oxidation product in 1 ml. of redistilled aniline was heated on the steam-bath for 30 minutes in a 10-ml. erlenmeyer flask. Dilution with water, adjustment of the *p*H to 4, and extraction with chloroform led to the recovery of 25 mg. of the unchanged permanganate oxidation product, m.p. 294–296° dec. (dark from 281°).[11] The identity was confirmed by the infrared spectrum.

(28) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 153.

Reaction of the Permanganate Oxidation Product with Diazomethane.—To a solution of 20 mg. of the permanganate oxidation product of haplophytine in 5 ml. of chloroform–ether was added 10 ml. of an ethereal solution of diazomethane.[29] The solution was allowed to stand for two hours and then the solvents were removed to leave a white powder, soluble in chloroform, ethanol and acetone. The residue was treated with hot ether and filtered; a reddish-brown, chloroform-soluble residue remained on the filter. The ethereal filtrate was dried over sodium sulfate and concentrated; after several hours a few milligrams of a beige powder separated from the solution and was collected, m.p. 215–225° dec. (dark from 180°).[11] The infrared absorption spectra of the material showed a strong absorption band at 1737 cm.$^{-1}$ and showed no absorption at 3150 cm.$^{-1}$ (present in the Nujol mull spectrum of the permanganate oxidation product). The band at 1150 cm.$^{-1}$ present in the spectrum of the permanganate oxidation product was considerably altered.

Attempted Reaction of the Permanganate Oxidation Product of Haplophytine with Alkaline Hydrogen Peroxide.—To a solution of 100 mg. of the permanganate oxidation product in 10 ml. of 5% sodium hydroxide solution was added 10 ml. of 30% hydrogen peroxide solution. The solution did not develop any color and only mild effervescence took place. After 2.5 hours a small amount of palladium-on-carbon was added to decompose the hydrogen peroxide and the mixture was filtered. The *p*H of the filtrate was adjusted to 7 with 2 *N* hydrochloric acid solution. The aqueous solution was extracted four times with 10-ml. portions of chloroform and the chloroform was removed by distillation from the combined chloroform layers; only a trace amount of residue was obtained. The *p*H of the aqueous solution was adjusted to 1 with 2 *N* hydrochloric acid solution and again extracted four times with 10-ml. portions of chloroform. The chloroform was removed from the combined layers and the residue was dissolved in 2 ml. of absolute ethanol. A dark solid separated slowly from the alcohol solution and was collected by vacuum filtration. The yield was 50 mg. The material was recrystallized from absolute alcohol to give a pink, semi-crystalline solid, m.p. 306–309° dec. (dark from 265°).[11] The infrared spectrum was almost identical to that of the permanganate oxidation product of haplophytine.

Treatment of Haplophytine with Hydrazine.—A 40-mg. quantity of haplophytine was heated for five minutes with 3 ml. of 40% hydrazine hydrate. Since the alkaloid did not dissolve completely, 4 ml. of ethanol was added, and the resulting solution was refluxed for one-half hour.[12] At the end of this time, the ethanol was removed, and the yellow solution was allowed to stand. Overnight there was deposited 20–30 mg. of colorless, fine needles, m.p. 270–280° dec.[22] One recrystallization from ethanol gave characteristic clumps of needles, m.p. 280–285° dec.,[22] which reacted with carbon tetrachloride in the presence of chloroform. The reaction with hydrazine hydrate was attempted several times, and was always negative.

In one experiment, 70–80 mg. of haplophytine was treated with 6–8 ml. of freshly-prepared anhydrous hydrazine.[30] The alkaloid did not dissolve immediately, but went into solution readily when warmed gently. However, the solution turned to a brilliant red as solution took place, the product rapidly darkened, and nothing crystalline could be isolated.

(29) F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 165.

(30) L. I. Smith and K. L. Howard, *Org. Syntheses*, **24**, 53 (1944).

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The Configuration of Cerebronic Acid

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The optical rotations of some derivatives of cerebronic acid have been compared with those of the corresponding derivatives of D-α-hydroxy acids. It is concluded that cerebronic acid has the D-configuration.

Cerebronic (phrenosinic) acid. one of the hydrolysis products of the sphingolipide cerebron (phrenosin), has been extensively investigated[1] since it was first isolated some fifty years ago[2]; more recent work has led to the recognition that cerebronic acid is in fact a mixture of the saturated straight chain C_{22}, C_{24} and C_{26} α-hydroxy acids, of which 2-hydroxytetracosanoic acid is presumably the preponderant component.[3] It is to be supposed, however, that the configurations of all three acids are the same, and that their optical properties are virtually indistinguishable. Therefore, an investigation of the configuration of cerebronic acid through application of the Displacement Principle (*vide infra*) ought still to retain the significance generally accorded results obtained by this method.

The Displacement Principle[4] (*Verschiebungssatz*), may be stated as follows[5]: analogous compounds of similar configuration undergo like shifts in rotation when similar substituents are introduced into the corresponding groups attached to the asymmetric center. A comparison of the rotations of some derivatives of cerebronic acid with those of the corresponding derivatives of D-lactic acid, D-mandelic acid and D-hexahydromandelic acid shows a gratifying over-all parallelism, the rotations becoming successively more positive as one progresses from the O-benzoyl methyl ester to the methyl ester. ethyl ester, amide and O-methyl ether methyl ester. The positive shift for the amide, although exemplifying only a special case of the Displacement Principle, is thought to constitute the most reliable diagnostic test for α-hydroxy acids belonging to the D-series; reference is made in that connection to the Amide Rule.[4,6] The pertinent results are summarized in Table I.

Additional support for our conclusions is based on the following considerations. It is known[7] that

(1) *Cf. e.g.*, H. Thierfelder and E. Klenk, "Die Chemie der Cerebroside und Phosphatide," J. Springer, Berlin, 1930.

(2) J. L. W. Thudichum, "Die Chemische Konstitution des Gehirns der Menschen und der Tiere," F. Pietzcker, Tübingen, 1901, p. 194 ff.

(3) R. Ashton, R. Robinson and J. C. Smith, *J. Chem. Soc.*, 283, 625 (1936); A. C. Chibnall, S. H. Piper and E. F. Williams, *Biochem. J.*, **30**, 100 (1936); D. M. Crowfoot, *J. Chem. Soc.*, 716 (1936); A. Müller and I. Binzer, *Ber.*, **72**, 615 (1939).

(4) K. Freudenberg in K. Freudenberg, "Stereochemie," F. Deuticke, Leipzig and Vienna, 1932, p. 693 ff.

(5) G. L. Jenkins and W. H. Hartung, "The Chemistry of Organic Medicinal Products," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 642.

(6) C. S. Hudson, THIS JOURNAL, **40**, 813 (1918).

(7) *E.g., cf.* G. W. Clough, *J. Chem. Soc.*, 2808 (1925).