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).

TABLE I[a]

Acid R—CHCOR″ (OR′)	[M]D R′ → Bz–, R″ → CH3O–	H–, CH3O–	H–, C2H5O–	H–, H2N–	CH3–, CH3O–
Cerebronic	+1.0 p	+10.4 p	+14 p[b]	+29.6 p	+74 p[b]
D-Mandelic[c]	−433 n	−237 m	−211 m	−144 pf	−173 a
D-Hexahydromandelic[d]	−38.6 n	−36.8 n	−24.4 n	+75.5 e	
D-Lactic[e]	−40.3 n	+8.6 n	+13.6 n	+22.3 w	+112.7 n

[a] The letters following the rotations refer to the solvent: n = no solvent; p = pyridine; m = methanol; pf = 1:1 pyridine–formic acid; a = acetone; e = ethanol; w = water. [b] P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, **52**, 227 (1922); P. A. Levene and P. S. Yang, *ibid.*, **102**, 541 (1933). The values here tabulated are averages of reported values. [c] K. Freudenberg and L. Markert, *Ber.*, **58**, 1753 (1925); P. Walden, *ibid.*, **38**, 345 (1905); G. W. Clough, *J. Chem. Soc.*, 2808 (1925); A. McKenzie and H. Wren, *ibid.*, 473 (1910). [d] K. Freudenberg and L. Markert, *Ber.*, **58**, 1753 (1925); C. E. Wood and M. A. Comley, *J. Chem. Soc.*, 2630 (1924). [e] T. Purdie and J. C. Irvine, *ibid.*, 483 (1899). K. Freudenberg and F. Rhino, *Ber.*, **57**, 1547 (1924); K. Freudenberg, F. Brauns and H. Siegel, *ibid.*, **56**, 193 (1923). Values from the last two references corrected to optical purity.

salts of D-α-hydroxy acids exhibit rotations more positive than those of the acids themselves. Cerebronic acid is levorotatory in chloroform but dextrorotatory in pyridine. This shift to a more positive value in going from chloroform to pyridine may be attributed to an increase in degree of ionization, and while a more direct study of this point was precluded by the virtual insolubility of alkali cerebronates, our thesis was borne out by a comparison with D-mandelic acid, whose rotation becomes increasingly positive as the solvent is changed in the order: chloroform, water, pyridine and aqueous sodium hydroxide. These results are summarized in Table II.

TABLE II

Acid	[M]D Chloroform	Water	Pyridine	Sodium salt in water
Cerebronic	−6.8[a]		+12.7	
D-Mandelic	−271	−240	−211	−179[b]

[a] E. Klenk, *Z. physiol. Chem.*, **174**, 214 (1928). [b] G. W. Clough, *J. Chem. Soc.*, 2808 (1925).

We conclude that the evidence now accumulated provides ample justification for assigning the D-configuration to cerebronic acid.[7a]

Experimental[8]

Cerebronic Acid.—The method of isolation of this substance was patterned in the main on the classical procedure of Klenk.[1] The hydrolysis of brain cerebroside mixture[9] gave a product mixture consisting principally of the acid and its methyl ester; saponification of this mixture yielded the sodium salt of cerebronic acid. The acid was purified through the magnesium salt and recrystallized from acetone. The material thus obtained had m.p. 90–93°, $[\alpha]^{34}D$ +3.3° (*c* 5.07, pyridine) (lit.[10] m.p. 91–93°, $[\alpha]D$ +3.55° (pyridine). The melting points reported in the literature[1] are somewhat variable, but the rotations are consistently near +3.4° (pyridine)).

Cerebronic Acid Methyl Ester.—A solution of 5.2 g. of the acid in 80 ml. of methanol was saturated with hydrogen chloride and heated under reflux for five hours. The solvent was stripped under vacuum and the residue taken up in ether. The ether layer was shaken with aqueous sodium carbonate, dried and stripped. The residue, after two recrystallizations from acetone, afforded 4.4 g. (85%) of the desired ester, m.p. 58–59°, $[\alpha]^{34}D$ +2.6° (*c* 5.34, pyridine) (lit.[11] m.p. 58° to 64°).

Cerebronic Acid Amide.—A solution of 2.0 g. of the methyl ester in 20 ml. of absolute ethanol was saturated with anhydrous ammonia at 0°. After standing for two days at room temperature, the reaction mixture was filtered and the residue recrystallized from methanol, yielding 0.55 g. (29%) of the desired amide, m.p. 118–119°, $[\alpha]^{46}D$ +7.7° (*c* 6.37, pyridine).

Anal. Calcd. for $C_{24}H_{49}O_2N$: C, 75.3; H, 12.78. Calcd. for $C_{26}H_{53}O_2N$: C, 75.9; H, 13.00. Found: C, 75.8; H, 12.73.

Cerebronic Acid Methyl Ester O-Benzoate.—Benzoyl chloride (1.0 g.) was added slowly to a solution of 0.70 g. of cerebronic acid methyl ester in a mixture of 2.1 ml. of anhydrous pyridine and 5 ml. of anhydrous ether. The mixture was kept near 0° during the addition. After having been allowed to stand for five hours, the product mixture was poured into ice-water, extracted with ether, and the ether layer washed with aqueous sodium carbonate and water. After drying over sodium sulfate, the ether layer was stripped and the residue recrystallized from methanol to give 0.13 g. (15%) of waxy crystals, m.p. 49–50°, $[\alpha]^{34}D$ +0.2° (*c* 5.04, pyridine), somewhat impure to judge from the analysis.

Anal. Calcd. for $C_{32}H_{54}O_4$: C, 76.6; H, 10.88. Calcd. for $C_{34}H_{58}O_4$: C, 76.9; H, 11.04. Found: C, 77.3; H, 11.88.

L(+)-Mandelic Acid.—The acid, resolved through the cinchonine salt,[12] had m.p. 132–133°, $[\alpha]^{34}D$ +149° (*c* 2.01 water), $[\alpha]^{35}D$ +131° (*c* 2.10, pyridine), $[\alpha]^{43}D$ +168° (*c* 1.23, chloroform). On the basis of the maximum rotation, $[\alpha]D$ 158° (water),[12] the optical purity of this acid is 94%, and, by extrapolation to 100% optical purity, the maximum rotations in the other solvents are $[\alpha]D$ 139° (pyridine) and $[\alpha]D$ 178° (chloroform).

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(7a) ADDED IN PROOF.—The optical properties of the long-chain (C_{10} to C_{18}) α-hydroxy acids derived from wool wax (D. H. S. Horn, F. W. Hougen, E. von Rudloff and D. A. Sutton, *J. Chem. Soc.*, 177 (1954)) bear a striking resemblance, in regard to sign and order of magnitude of rotation, to those of cerebronic acid; we think it likely that these acids also possess the D-configuration.

(8) Microanalyses by W. Manser (Zürich).

(9) Bios Laboratories, Inc., New York, N. Y.

(10) P. A. Levene and C. J. West, *J. Biol. Chem.*, **26**, 115 (1916).

(11) P. A. Levene and C. J. West, *ibid.*, **15**, 193 (1913); P. A. Levene and P. S. Yang, *ibid.*, **102**, 541 (1933).

(12) A. McKenzie, *J. Chem. Soc.*, 964 (1899).