of methanol and warmed at 50° for 6 hr. under a Dry Ice condenser. The solvent and excess dimethylamine were then removed by evaporation. The residue was shaken with 1000 ml. of 5% aqueous sodium hydroxide and the mixture extracted with ether. After drying the extract and evaporating the ether, the residue was distilled, yielding 96 g. (64%) of decyldimethylamine, b.p., 103°/10 mm., n_D^{25} 1.4297.

Anal. Calcd. for $C_{10}H_{27}N$: N, 7.56. Found: N, 7.63.

Docosyldimethylamine. Docosyl bromide[8] 50 g., 0.13 mole, and dimethylamine, 39 g., 0.9 mole, were dissolved in 260 ml. of ether, sealed in glass tubes and warmed at 50° for 18 hr. The reaction mixtures were then removed from the tubes, combined and extracted with 250 ml. of 5% aqueous sodium hydroxide. The bulk of the excess dimethylamine boiled off during this operation. After drying the extract, the ether was evaporated yielding 38 g. of a pale yellow waxy mass which resisted all attempts to recrystallize it. Its hydrochloride was equally intractable. The crude base was used, therefore, in the quaternization step.

2-Bromoethyl(substituted)ammonium bromides The appropriate tertiary amine was dissolved in a tenfold excess of ethylene bromide and treated as recorded in Table II. In several cases, addition of methanol was required to affect homogeneity. The excess ethylene bromide and solvent were then removed in a flash evaporator *in vacuo* at 40°. The crystalline residue was washed with cold ethyl acetate, collected, and dried and stored out of contact with moisture. These materials were of sufficient purity for use directly in the next step (the docosyldimethyl derivative is an exception). Analytical samples were prepared by recrystallization from the solvents shown in Table II.

Taurine betaines. The appropriate 2-bromoethyl(substituted)ammonium bromide was treated in aqueous solution (the docosyldimethyl derivative required 25% ethanol) with a 5% molar excess of sodium sulfite for 5–10 hr at 85°. The reaction mixture was then concentrated to a moist residue in a flash evaporator and triturated with 1 l. of cold concd. hydrochloric acid per mole of quaternary used in the reaction. Just as with taurine,[9] the taurine betaines are soluble in this medium while the inorganic salts are only slightly soluble. The trituration mixture was filtered through a sintered glass funnel and the clear filtrate was concentrated *in vacuo* at 50° to a thick sirup. Addition of ethanol or isopropyl alcohol caused the taurine betaine to precipitate. In the cases of the decyl, dodecyl, and docosyl derivatives, the concentration and trituration steps were unnecessary because the products precipitated directly from the reaction mixtures on cooling.

The crude products were recrystallized from the solvents listed in Table III.

The infrared spectra of the products, measured with a Perkin-Elmer Model 21 spectrophotometer, contained strong bands near 8.4 and 9.6 μ characteristic of the sulfonate group.

Titrations with acid and base. Samples of each taurine betaine were titrated in aqueous or aqueous ethanolic solution with 0.1*N* hydrochloric acid and with 0.1*N* sodium hydroxide with the aid of a *p*H meter. No buffer capacity was observed between *p*H 3 and 11.

Conductivity. The specific conductance of 0.25% solutions of three taurine betaines was determined at 25° using a Henry cell and a Leeds and Northrup catalog No. 4866 conductance bridge (60 cycles/sec). The results are shown in Table I together with the value for a typical strong electrolyte, potassium chloride.

Solubility. Solubility was determined at 30° in a constant temperature bath. Suspensions of taurine betaines in 0.5 g. of water were prepared and diluted dropwise with water at 24-hr. intervals until the solids dissolved. The solubilities are recorded in Table I.

Acknowledgment. The author wishes to express his appreciation to Mr. E. W. Blank and staff for the microanalyses and infrared measurements and to Mr. Conrad Jakob for the conductivity and solubility measurements.

JERSEY CITY, N. J.

(8) J. von Braun *et al.*, *Ann.*, **472**, 121 (1929).

(9) F. Cortese, *Org. Syntheses*, Coll. Vol. II, 564 (1943)

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WAYNE STATE UNIVERSITY]

The Structure of Helvolic Acid. III[1,2]

NORMAN L. ALLINGER AND JAMES L. COKE[3]

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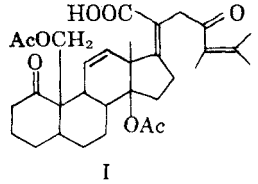
Chemical and spectroscopic studies, taken in conjunction with earlier data, have led to a tentative formulation of the structure of helvolic acid as that shown in VI.

Helvolic acid, an antibiotic isolated from the mold *Aspergillus fumigatus*, has been the subject of fairly extensive chemical and biological studies.[4] Attempts to summarize the available data in 1956 yielded a tentative structure (I)[1,4] for the compound.



I

The structure of helvolic acid has now been further investigated, and additional evidence has been obtained. While the actual structure still has not been established with certainty, a good deal more has been learned about it.

The empirical formula deduced earlier is still regarded as correct, as is the presence of the indi-

(1) Paper II, N. L. Allinger, *J. Org. Chem.*, 21, 1180 (1956).

(2) This research was supported by a grant (E-2267) from the U. S. Public Health Service, National Institutes of Health.

(3) Ethyl Corp. Research Fellow, 1958–1959.

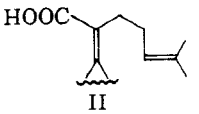
(4) For a summary of the earlier literature and references, see D. J. Cram and N. L. Allinger, *J. Am. Chem. Soc.*, 78, 5275 (1956).

cated functionality: an α,β-unsaturated acid, an α,β-unsaturated ketone, a saturated ketone, two acetoxyls, and an isolated double bond. The presence of a steroid nucleus was suggested, but not really proven, by the earlier selenium dehydrogenation study.[4] On the assumption that the molecule contains a steroid nucleus, and none of the subsequent work appears inconsistent with this hypothesis, the problem is essentially reduced to locating the described functionality in the ring system.

The side chain. The side chain proposed earlier (I) contains some unusual features, especially the α,β-unsaturated acid grouping, and the oxygen at C-23. The latter was placed as shown because helvolic acid yielded acetone upon ozonolysis, while "dihydrohelvolic acid," in which the double bond conjugated with the ketone had been reduced (from the ultraviolet spectrum), did not. Subsequent investigation has shown that the isolated double bond and the one conjugated with the ketone are reduced at comparable rates, and the "dihydrohelvolic acid" is in fact a mixture of products. The failure to isolate acetone from its small scale ozonolysis is therefore of little significance. The ozonolysis of helvolic acid itself has now been repeated on large scale (200 mg.) and succinic acid was isolated and identified by comparison with an authentic sample.

Tetrahydrohelvolic acid is a definite single compound in which both the isolated double bond and the one conjugated with the ketone in helvolic acid have been saturated. Ozonolysis of this compound proceeded slowly and gave a mixture from which neither acetone nor succinic acid was isolated. Instead a volatile fatty acid, the presence of which was easily detected by odor, was isolated as its *p*-toluide derivative. No such odor was detected upon the ozonolysis of helvolic acid itself. The *p*-toluide, m.p. 71.5°, appeared from the carbon-hydrogen and C-methyl analyses to be a derivative of 5-methylhexanoic acid. An authentic sample of this acid was, therefore, synthesized in several steps beginning with isoamyl bromide and malonic ester. The infrared spectra of the *p*-toluides of the synthetic and natural acids were identical, the compounds had the same melting point and they gave no mixture melting point depression.

These data lead to a formulation for the side chain as shown in II.

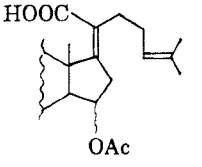


II

The first acetoxyl. The C-14 acetoxyl was suggested earlier since such structures are common in nature and since the acetoxyl appeared to be somewhat hindered and located somewhere in the vicinity of the α,β-unsaturated acid. Although no very strong evidence for its location was or is available, another location now seems preferable.

When helvolic acid was heated above its melting point it lost acetic acid and carbon dioxide.[4] The product of the reaction, which was designated earlier as "pyrohelvolic acid," was amorphous but showed characteristic ultraviolet absorption maxima for a homocyclic conjugated diene at 299, 287, and 275 mμ, with extinction coefficients of 6200, 8200, and 6900, respectively. When methyl helvolate was pyrolyzed, even with added acid catalyst, no elimination of either carbon dioxide or acetic acid occurred and the starting material was recovered unchanged. The reaction consequently appears to be a decarboxylative elimination.

When tetrahydrohelvolic acid was pyrolyzed, acetic acid and carbon dioxide were lost as with helvolic acid itself, but the "pyrotetrahydrohelvolic" acid obtained showed no evidence for a conjugated homocyclic diene. The ultraviolet spectrum had λ_{max} 221 mμ, ϵ 6900; therefore, one of the double bonds hydrogenated in going from helvolic acid to tetrahydrohelvolic acid is necessary for the formation of the conjugated homocyclic diene, but not for the elimination reaction. This suggests that the diene formation is subsequent to the elimination, and these reactions can be formulated by a reasonable mechanistic sequence by assigning helvolic acid the partial structure III. Decarboxylative eliminations are well known, the one involved in this case appears to be a vinylogous variety. The structure III adequately explains both the failure of the ester to undergo the pyrolysis reaction, and the spectra of the various products.



III

The second acetoxyl and saturated ketone. Very mild basic hydrolysis converted helvolic to hevolinic acid, in which an acetate had been hydrolyzed to yield an alcohol with a very intense O—H stretching band in the infrared spectrum. Earlier,[4] these facts were interpreted as the 1-keto-19-acetoxyl feature. That this interpretation is incorrect is shown by the fact that although helvolinic acid decomposed quite readily in base, no formaldehyde could be detected in the reaction mixture. The NMR spectrum is also inconsistent with a primary acetoxyl.

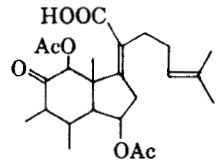
One peculiar fact which remains unexplained up until this point is the unusually high wave length (318 mμ) of the n$\rightarrow\pi^*$ transition in octahydrohelvolic acid. The absence of any absorption at lower wave length (end absorption, λ_{220} ϵ 670) showed that this ketone could not be conjugated. The strongly negative Cotton effect curve of methyl hexahydrohelvolate showed a trough at 346 mμ,

which is also unusually high for an unconjugated ketone, but consistent with the ultraviolet spectrum.

The only obvious arrangement of groups which might account for these spectral data would be to have an axial acetoxyl on the carbon adjacent to the ketone. The presence of such an acetoxyl should shift the ultraviolet maximum about 10 mμ toward longer wave length, and similarly shift the trough of the rotatory dispersion curve by 15 mμ.[5] Such a structure is also consistent with the unusually large extinction coefficient of octahydrohelvolic acid in the ultraviolet (ε 52) and the exceedingly large amplitude[6] of the Cotton effect curve ([α] = 23,000 for methyl hexahydrohelvolate). As the sign of the Cotton effect curve is negative, the octant rule[7] leads to the conclusion that the α-acetoxyketone grouping cannot be a ring B. A 12-keto-11β-acetoxy or an 11-keto-12α-acetoxy structure would be consistent with the spectral data, as would various arrangements in ring A.

Further evidence for the α-acetoxy ketone structure and its location was obtained as follows. If the proposed structure were present in helvolic acid, then helvolinic acid would be an α-ketol. It was found that while helvolic acid itself gave a negative acyloin test with bismuth oxide, helvolinic acid gave a positive test. A mild chromic acid oxidation of tetrahydrohelvolinic acid gave a substance which was tentatively identified as an α-diketone. It was a yellow crystalline compound which gave a positive ferric chloride test and showed λ_{max} 283 mμ, ε 880. Upon addition of base the ultraviolet spectrum changed to λ_{max} 309 mμ, ε 2500.[8] These values are consistent with an 11,12-diketo structure,[9] but are not consistent with the diketone in ring A, as compounds of the latter type exist almost completely in the enol form.[10]

As helvolic acid is hydrolyzed to helvolinic acid very easily, and as helvolinic acid can be acetylated to give helvolic acid under mild conditions, an 11β-acetoxyl seems most unlikely. The part structure containing the α-acetoxy ketone function seems likely therefore to be an 11-keto-12α-acetoxy system, summarized as IV. Strong support for this assignment is also available from the NMR spectra (see below).



IV

The α,β-unsaturated ketone. The proton magnetic resonance spectrum of helvolic acid (see below) showed unequivocally that the compound contains the unit R′—CH=CH—COR, and further it showed that the carbon of R′ attached to the olefinic carbon did not carry any hydrogens. As this grouping was not in the side chain, it could only be located in ring A. The NMR spectrum would be quite consistent with a Δ^1-3-keto structure. It would not be consistent with any other steroidal structure that might reasonably be expected to be found in nature except possibly a Δ^2-1-keto-4,4-*gem*-dimethyl steroid. Biogenetic considerations certainly favor the Δ^1-3-keto structure, and the 4,4-*gem*-dimethyl structure can be eliminated on chemical grounds (see below).

The ultraviolet spectrum of this grouping (λ_{max} 236 mμ, ε 10,600) is not in good agreement with this assignment, but as this spectrum is obtained as the difference between two experimentally determined spectra, it is subject to sizeable error.

The rotatory dispersion curves of methyl tetrahydrohelvolate and methyl hexahydrohelvolate were also useful for confirming the stereochemistry at C-5. As the former contains the carbonyl (which was α,β-unsaturated but in which the olefinic linkage has now been reduced) while the latter compound has this carbonyl reduced to an alcohol, the difference between these two rotatory dispersion curves gave the curve characteristic of the lone carbonyl (Fig. 1). A positive Cotton effect for this group was noted, which is qualitatively consistent with the 5α but not the 5β epimer.[11] The rotation of this keto group in helvolic acid was +680° at the peak, and the corresponding value for 3-cholestanone is +810°.

Methyl tetrahydrohelvolate was brominated with pyridine hydrobromide perbromide to give a crystalline monobromo derivative. This bromo compound was dehydrobrominated with collidine, and the resulting α,β-unsaturated ketone grouping (resolved graphically from the remaining absorption) showed λ_{max} 236 mμ, identical to that of methyl helvolate and consistent with the Δ^1-5α structure.

Helvolic acid contains thirty-two carbons, four of which make up the acetates. Relative to cholesterol, to which it appears closely related in its carbon skeleton, there is an "extra" methyl group. Kuhn-Roth determinations on helvolic acid and its tetra- and octahydro derivatives indicated approximately six C-methyl groups, which were

(5) C. Djerassi, *Optical Rotatory Dispersion: Applications to Organic Chemistry*, McGraw-Hill, New York, 1960, p. 114.

(6) For nomenclature see: (a) N. L. Allinger, J. Allinger, L. E. Geller, and C. Djerassi, *J. Org. Chem.*, 25, 6 (1960); (b) C. Djerassi and W. Klyne, *J. Chem. Soc. (Proc.)*, 55 (1957).

(7) Ref. 5, p. 178.

(8) C. Djerassi, R. Mauli, and L. H. Zalkow, *J. Am. Chem. Soc.*, 81, 3424 (1959).

(9) For example: O. Schindler and T. Reichstein, *Helv. Chim. Acta*, 36, 1007 (1953); 37, 667 (1954); J. Barnett and T. Reichstein, *Helv. Chim. Acta*, 21, 926 (1938).

(10) E. T. Stiller and O. Rosenheim, *J. Chem. Soc.*, 353 (1938); L. Ruzicka, P. A. Plattner, and M. Furrer, *Helv. Chim. Acta*, 27, 524 (1944).

(11) Ref. 5, p. 42, 50.

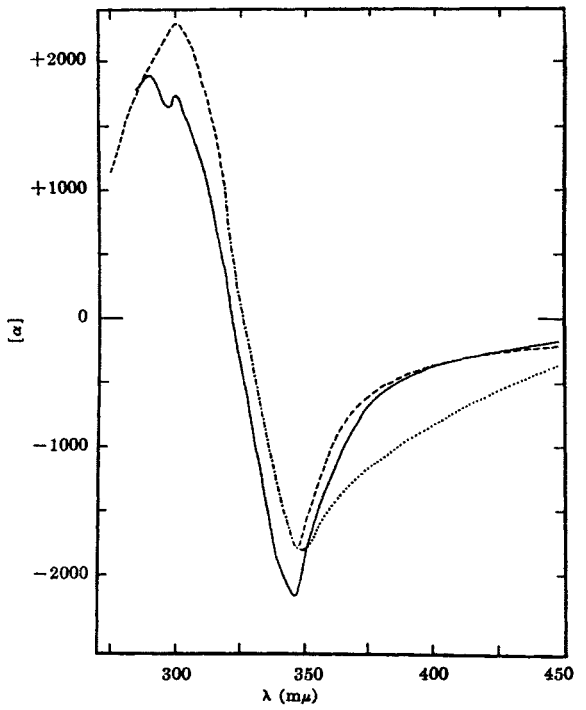


Fig. 1. Rotatory dispersion curves. Methyl helvolate .....; methyl tetrahydrohelvolate – – – –; methyl hexahydrohelvolate ———; (methanol, 25°, c = 0.1)

pictured as the two acetoxyls, the isopropylidene, the two angular methyls, and the "extra" methyl.

It was suggested earlier[1] on the basis of NMR data that the extra methyl was on a double bond, and it was tentatively placed at C-24. The degradative work shows the methyl is in fact not in the side chain, and it must therefore be in the ring system. A redetermination of the NMR spectrum has been made with modern instrumentation, and it is now clear that the methyl is not located on a double bond. The spectrum obtained earlier, which appears to have been the first recorded attempt to apply NMR to the steroid field,[12] was inadequate because of the rather primitive equipment then available.

Known naturally occurring compounds[13,14] which contain "extra" methyls in the ring system often have them located at C-4, and this possibility was considered in the present case. Helvolic acid did not form a furfurylidene adduct, which indicated there were no unhindered methylene groups adjacent to ketones in the molecule. Tetrahydrohelvolic acid formed only a monofurfurylidene adduct, consistent with a methylene at C-2.

A 4-*gem*-dimethyl grouping was ruled out by the fact that, when methyl tetrahydrohelvolate was reduced with sodium borohydride and the resulting material was treated with phosphorus pentachloride, a product was obtained which gave no acetone upon ozonolysis.

A 4β-methyl can be ruled out by the fact that helvolic acid can be hydrolyzed to helvolinic acid with base, and the helvolinic acid can be reacetylated to give back helvolic acid, and both steps proceed in good yield. A 4β-methyl would be expected to epimerize under these conditions.

When the rotatory dispersion curve of methyl tetrahydrohelvolate was determined in methanol and a trace of hydrochloric acid was added, hemiketal formation took place to the extent of about 14%. This fact means the ketone is hindered in some way, and is consistent with a 4α-methyl-3-ketone.[13,15] Further evidence for this structure was sought from molecular rotation differences. Methyl hexahydrohelvolate is assigned the 3β-ol structure, the corresponding acetate and ketone (methyl tetrahydrohelvolate) are known, and the molecular rotation differences can be compared with those found with a 5α-3β-ol and a 5α-4α-methyl-3β-ol. The values in the helvolic acid series are between the methylated and unmethylated compounds (Table I), but are in fair agreement with the structure suggested (V).

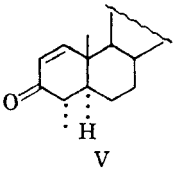
TABLE I

MOLECULAR ROTATION DATA[a]

Configuration	Δ_1	Δ_2
5α-3β-ol[b]	−37	+66
5α-4α-Methyl-3β-ol[c]	+64 to +78	−39 to +39
Methyl hexahydrohelvolate	+35	+47

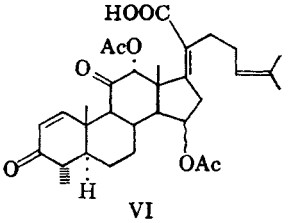
[a] $\Delta_1 = [M]_{OAc} - [M]_{OH}$; $\Delta_2 = [M]_{C=O} - [M]_{OH}$. [b] L. F. Fieser and M. Fieser, *Steroids*, Reinhold Publishing Corp., New York, 1959, p. 179. [c] Ref. 13 and 14.

The rotatory dispersion arguments used to arrive at the 3-keto-5α-structure are qualitatively equally valid with the 4α-methyl derivative.



V

At least a tentative assignment for the structure of helvolic acid can now be made by combining the available part structures. The proposed structure is VI.



VI

(12) P. Bladon, *Ann. Reports*, 53, 217 (1956).

(13)(a) C. Djerassi, G. W. Krakower, A. J. Lemin, L. H. Liu, J. S. Mills, and R. Villotti, *J. Am. Chem. Soc.*, 80, 6284 (1958); (b) G. W. Krakower, Ph.D. thesis, Wayne State University (1958).

(14) Y. Mazur, A. Weizmann and F. Sondheimer, *J. Am. Chem. Soc.*, 80, 6293 (1958).

(15) Ref. 5, p. 146.

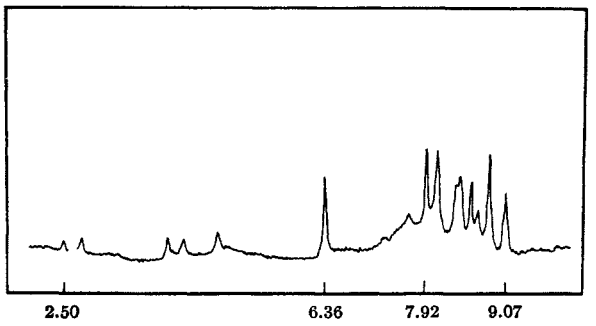
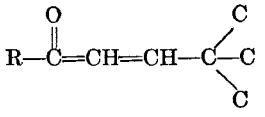


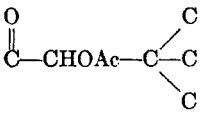
Fig. 2. The proton magnetic resonance spectrum of methyl helvolate

NMR Spectra. The NMR spectrum of methyl helvolate is shown in Fig. 2. The ratio of the sum of the areas of the peaks due to methyl groups to the area due to the methyl of the methyl ester gives the number of methyl groups per molecule as eight in methyl helvolate and methyl tetrahydrohelvolate, seven in helvolic acid, and nine in methyl hexahydrohelvolate acetate.[16] The total number of methyl groups in helvolic acid is therefore thought to be seven, or one more than indicated by Kuhn-Roth oxidation, and this is consistent with structure VI. The assignment of the peaks in the NMR spectra were made as follows. For helvolic acid and methyl helvolate, the pair of doublets centered at 2.62 and 4.13 p.p.m. with J = 5.2 cycles/sec. show conclusive evidence for the part structure VII. There are very few ways such a structure



VII

can be accommodated in the steroid ring system, and these peaks are assigned to the protons at C-1 and C-2, respectively.[17] The location is similar to that found for a Δ^1-3-ketone by Shoolery and Rogers (2.17 and 4.00),[18] and the splitting is about as expected. It is seen, however, that the area under the doublet at 4.13 is approximately twice as great as that under the doublet at 2.62. In methyl tetrahydrohelvolate, as the double bond at C-1 has been saturated, the resonance at 2.62 is gone. The resonance at 4.13 is still present, however, but reduced to half its former intensity and shifted slightly to 4.15 (J = 5.2 c.p.s.). The remaining doublet, which fortuitously coincided exactly with the C-2 proton, is in a region consistent with it being due to a proton on a carbon carrying an acetoxyl, and it is split by a neighboring proton. This absorption is assigned to the proton at C-15. The proton at C-15 may couple effectively only with one of the protons at C-16, especially if the acetoxyl group is β. The single proton peak at 4.74 is present in all the compounds examined, is unsplit, is located in the proper region to be equatorial on a carbon carrying an acetoxyl, and is indicative of part structure VIII. This resonance frequency is assigned to the C-12β proton.



VIII

The peak at 6.36 in methyl helvolate corresponds in area to three protons, is not present in helvolic acid, and is assigned to the methyl ester. The peak at 7.92 and 8.06 were assigned to the methyl groups of the two acetoxyls. In methyl hexahydrohelvolate acetate, the 7.92 peak had about twice the intensity of the 8.06 peak, and was assigned, in part, to the 3β-acetoxyl.

The doublet at 8.36 and 8.41 in methyl helvolate corresponds in area to six hydrogens. As the methyls in the side chain (C-26 and C-27) are not equivalent, they are expected to give a doublet in this region. The tetrahydro compound and other reduced derivatives show no resonance in this region, but increased resonance at higher field. This doublet is therefore assigned to the protons on C-26 and C-27.

The remaining resonance due to methyl groups in methyl helvolate consisted of small peaks at 8.58 and 8.72 and larger peaks at 8.87 and 9.07. The most obvious assignment was the C-4 methyl to the first two peaks and the protons of C-19 and C-18 respectively to the last two. The difficulty with this assignment is that 8.87 seems to be too high a field for the C-19 grouping in the proposed structure. Shoolery and Rogers report the C-19 methyl for a Δ^1-3,11-dione at 8.67.[18]

If the peak at 8.58 is assigned to the C-19 protons, then the C-4 methyl group has to be assigned to the doublet at 8.72 and 8.87. The disparity in size between these components is so great that such an assignment would seem reasonable only if still another methyl were also contributing to the absorption at 8.87. While such a situation might be possible, no other evidence for such an "extra" methyl has been found. No trace of the olefinic hydrogen at C-24 in methyl helvolate could be seen.

Methyl tetrahydrohelvolate showed a peak corresponding to about three methyls at 8.68, one corresponding to about two methyls at 9.07, and very small peaks at 8.77, 8.88, and 9.16. The hexahydro and octahydro compounds were similar in this region. The detail did not appear sufficient to warrant further conclusions, however.

(16) These numbers may be in error by ±1.

(17) All field strengths are given in parts per million relative to tetramethylsilane (tau values), see G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(18) J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958). An exact correspondence is not expected as their spectra and ours were determined under different experimental conditions.

The NMR spectrum of the amorphous material obtained by treating "pyrohelvolic acid" with diazomethane was also obtained, but was of poor quality. It could be seen, however, that one of the acetoxyl groups had been lost, and the resonance at 8.36–8.41 in helvolic acid was gone. Clearly there were no vinyl methyls remaining. There was no resonance which could be attributed to the methyl of the ester group present, and hence the material earlier called pyrohelvolic acid actually appeared to be a substance which had been decarboxylated.

The similarity in biological and some chemical properties of helvolic acid and cephalosporin P was noted by earlier workers.[19] An independent investigation of the structure of cephalosporin P has been carried out by the group at Oxford University. The structure they have assigned to cephalosporin shows many similarities to VI, but many differences also.

EXPERIMENTAL

Helvolic acid. The material used in this investigation was part of a batch described earlier.[4] After crystallization from aqueous methanol it had a m.p. of 208–212° dec.; reported[4] m.p. 211.3–212.1° dec.

Anal. Found: 13.12% or 1.69 O—Ac/mole. Found: 10.95% or 4.05 C—CH_3/mole.

Tetrahydrohelvolic acid. This compound was prepared as described earlier,[4] m.p. 192–193° dec. (reported[4] m.p. 195–196.5° dec.).

Anal. Found: 10.05% or 1.31 O—Ac/mole. Found: 9.55% or 3.56 C—CH_3/mole.

Tetrahydrohelvolinic acid furfurylidene. A solution of 80 mg. of tetrahydrohelvolic acid, 41.4 mg. of freshly distilled furfural, 2.80 ml. of 0.105*N* sodium hydroxide, and 2 ml. of ethanol was allowed to stand at room temperature in the dark for 94 hr. The solution was then diluted with water and acidified with hydrochloric acid. The precipitate was collected, and after two crystallizations from methanol furnished 62 mg. of material, m.p. 222–222.5° dec. The infrared spectrum showed a strong sharp hydroxyl band at 2.99 μ, a broad carboxyl band at 3–4 μ, carbonyl absorption from 5.75 to 5.90 μ, and a sharp band at 6.23 μ. The ultraviolet spectrum had λ_{max} 327 mμ, ϵ 19,300.

Anal. Calcd. for $C_{35}H_{46}O_8$: C, 70.69; H, 7.80. Found: C, 70.68; H, 7.89.

Ozonolysis of tetrahydrohelvolic acid. A 100-mg. sample of tetrahydrohelvolic acid in 60 ml. of purified methylene chloride was treated with ozone at −70° for 1.5 hr. The solution was then warmed to room temperature and stirred with 30 ml. of 30% hydrogen peroxide for 3 hr. The excess peroxide was then decomposed by adding a trace of platinum black and stirring the mixture for 8 hr. The solution was filtered, acidified with dilute sulfuric acid and steam distilled into 20 ml. of 20% aqueous sodium hydroxide until the distillate was odorless. The organic phase in the distillate was separated and discarded. The aqueous phase was acidified with 10 ml. of concd. hydrochloric acid and extracted three times with ether. The ether extracts were combined, dried, and concentrated to a small volume with the aid of a small Vigreux column. The resulting liquid was heated under reflux for 1.5 hr. with 1 ml. of thionyl chloride. A solution of 1 g. of *p*-toluidine in 10 ml. of benzene was then added and the mixture was heated under reflux for 10 min. The cooled mixture was washed successively with water, dilute acid, dilute base, and water. The benzene was evaporated, and the residue consisted of 14.2 mg. of crude toluide. This material was chromatographed on alumina and then crystallized from pentane, m.p. 70–71.5°.

Anal. Calcd. for $C_{14}H_{21}NO$: C, 76.65; H, 9.62. Found: C, 76.18; H, 9.52; 7.73% or 1.14 C—CH_3/mole.

An authentic sample of isoheptanoic acid *p*-toluide was prepared in several steps beginning with isoamyl bromide and malonic ester. This material had a m.p. of 72–72.5° (literature[20] 74°), and gave a value of 6.8%, or 1.00 C—CH_3/mole. This compound gave no mixture melting point depression with the compound from the ozonolysis, and the two compounds had identical infrared spectra.

Ozonolysis of helvolic acid. A solution of 200 mg. of helvolic acid in 50 ml. of purified methylene chloride was treated with ozone for 1.5 hr. at −70°. The mixture was then warmed to room temperature and stirred for 11 hr. with 30 ml. of 30% hydrogen peroxide. The excess hydrogen peroxide was then destroyed by stirring with platinum black. The platinum black was removed by filtration and the clear filtrate was extracted continuously with methylene chloride for 67 hr. The methylene chloride solution was dried and the solvent was evaporated. The residue was taken up in 2 ml. of chloroform and allowed to stand at room temperature for 3 hr. The crystalline material which formed was removed by suction filtration and dried to yield 1 mg. of material which sublimed at 146° and had a m.p. of 180–182°. An authentic sample of succinic acid sublimed at 142° and had a m.p. of 179–182°. The crystals of the two samples were identical in shape. A mixture melting point showed sublimation at 145° and a m.p. of 178–182°. The infrared spectra of the two materials were identical. At no time during this experiment was the strong characteristic odor of isoheptanoic acid detected.

Methyl bromotetrahydrohelvolate.[21] A solution of 49.4 mg. of pyridine hydrobromide perbromide in 5 ml. of purified tetrahydrofuran was added dropwise with shaking to a solution of 84 mg. of methyl tetrahydrohelvolate in 5 ml. of purified tetrahydrofuran. The mixture was allowed to stand for 10 min. and was then diluted with 45 ml. of water. The solid which formed was removed by suction filtration and was crystallized from aqueous methanol to yield 73 mg. of methyl bromotetrahydrohelvolate, m.p. 240–242°. The infrared spectrum of this product was indistinguishible from that of the starting material.

Anal. Calcd. for $C_{33}H_{47}O_8Br$: C, 60.82; H, 7.27. Found: C, 60.97; H, 7.47.

Dehydrobromination of methyl bromotetrahydrohelvolate.[22] A solution of 65 mg. of methyl bromotetrahydrohelvolate in 2 ml. of collidine was heated at reflux for 2 hr. The mixture was cooled and allowed to stand overnight at room temperature. The mixture was taken up in ether and washed several times with dilute hydrochloric acid to remove the collidine. The ether solution was washed with water, dried and the ether was removed on a steam bath. The gummy residue, after chromatography on Brockman III alumina, yielded 4 mg. of crystalline material, m.p. 234–238°, in addition to a large amount of gummy material. The ultraviolet spectrum found by subtracting the spectrum of methyl tetrahydrohelvolate from the spectrum of this crystalline material showed λ_{max} 236.5 mμ, ϵ 3,200. The low ϵ value indicated the material was still somewhat impure but it was not obtained in a quantity sufficient for extensive purification. The infrared spectrum showed carbonyl bands at 5.68–5.80 and 5.95 μ and was nearly identical to that of methyl helvolate.

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Acidic reduction of helvolic acid. A solution of 200 mg. of helvolic acid in 25 ml. of glacial acetic acid was hydrogenated with 30 mg. of platinum oxide at atmospheric pressure and room temperature until 2 moles (18.4 ml.) of hydrogen was absorbed. The mixture was filtered to remove the catalyst and the solvent was removed under vacuum. The residue gave, after crystallization from aqueous methanol, 154 mg. of tetrahydrohelvolic acid, m.p. 202–204° dec. (reported[3] 195–196.5°[2]). A mixture melting point with material obtained by reduction of helvolic acid in methanol showed no depression.

Esterification of a sample of the material obtained in this reduction, using diazomethane in methylene chloride, gave methyl tetrahydrohelvolate which on crystallization from aqueous methanol showed a m.p. of 210–212.5° (reported[1] m.p. 208.2–208.9°[1]). A mixture melting point with authentic methyl tetrahydrohelvolate, m.p. 209.5–212°, showed no depression.

Oxidation of methyl hexahydrohelvolate.[23] A solution of 4.64 mg. of chromium trioxide in 2 ml. of glacial acetic acid was added slowly to a solution of 40 mg. of methyl hexahydrohelvolate in 2 ml. of glacial acetic acid. The solution was allowed to stand at room temperature for 2.3 hr. and was then diluted with water. The solid which formed was removed by suction filtration and crystallization from aqueous methanol gave 26 mg. of crystalline material, m.p. 207–208.5°. Mixture melting point with authentic methyl tetrahydrohelvolate (m.p. 209.5–212°) showed a m.p. of 205–208.5°. The infrared spectrum of the product and authentic material were nearly identical.

Helvolinic acid.[4] A 100-mg. sample of helvolic acid was dissolved in 5 ml. of 0.1*N* sodium hydroxide and the solution was warmed to 40° for 5 min. The solution was then cooled and acidified with dilute hydrochloric acid. The solid which formed was removed by suction filtration and two crystallizations from aqueous methanol gave 72 mg. of helvolinic acid, m.p. 196–197° dec. (reported[1] m.p. 196–197.5°[1]). Helvolinic acid gave (contrary to an earlier report[4]) a positive bismuth oxide test for an acyloin group.

Anal. For *O*—acetate. Found: 5.96% or 0.71 OAc/mole.

Bismuth oxide oxidation of helvolinic acid.[24,25] A mixture of 26 mg. of helvolinic acid, 23.6 mg. of bismuth oxide and 2 ml. of glacial acetic acid was heated to 105–115° with efficient stirring for 1 hr. under nitrogen. The mixture was cooled, filtered to remove all solids and water was added. The crystalline material which formed was collected by suction filtration and dried to yield 15 mg. of slightly yellow material, m.p. 209–210° dec. This material gave a positive ferric chloride test for enols.

Bismuth oxide oxidation of tetrahydrohelvolinic acid.[24,25] A mixture of 76 mg. of tetrahydrohelvolinic acid and 76 mg. of bismuth oxide in 6 ml. of glacial acetic acid was heated at 105–115° with efficient stirring for 1 hr. under nitrogen. The mixture was cooled, filtered, and diluted with water. The solid which formed was collected by suction filtration. Precipitation from aqueous methanol yielded 67 mg. of amorphous solid, m.p. 126–138° dec. This material gave a positive ferric chloride test for enols. The infrared spectrum showed fairly strong hydoxyl absorption at 2.97 μ, broad carbonyl absorption at 5.77–5.84 μ and a weak band at 6.06 μ. The ultraviolet spectrum showed λ_{max} 220 mμ, ϵ 8,000, λ_{max} 283 mμ, ϵ 880 which shifted to λ_{max} 308 mμ, ϵ 2,500 on addition of a drop of base.

Chromium trioxide oxidation of tetrahydrohelvolinic acid. A solution of 2.2 mg. of chromium trioxide in 1 ml. of glacial acetic acid was added slowly to a solution of 17 mg. of tetrahydrohelvolinic acid in 1 ml. of glacial acetic acid. The resulting solution was allowed to stand at room temperature for 3.3 hr. and was then diluted with water. The solid material which formed was collected by suction filtration and crystallization from aqueous acetic acid gave 11.5 mg. of slightly yellow crystalline material m.p. 196–199° dec. This material gave a positive ferric chloride test for enols. The infrared spectrum of this material was identical with that of the product obtained by bismuth oxide oxidation of tetrahydrohelvolinic acid except that this product showed only a trace of hydroxyl absorption at 2.95 μ.

Octahydrohelvolic acid. A solution of 200 mg. of helvolic acid in 25 ml. of glacial acetic acid was hydrogenated with 200 mg. of platinum oxide at atmospheric pressure and room temperatures until 4 moles (36.8 ml.) of hydrogen had been absorbed. The catalyst was removed by filtration and the solution was concentrated and diluted with water. The solid which formed was collected by suction filtration and crystallized four times from aqueous acetic acid to yield 92 mg. of octahydrohelvolic acid, m.p. 207–212° (reported[4] m.p. 214–220°).

Anal. For O—acetate: Found: 10.60% or 1.39 O—Ac/mole. For C-methyl: Found: 9.41% or 3.54 C—CH_3/mole.

A 10-mg. sample of octahydrohelvolic acid was heated at 250° for 15 min. at 10 mm. The glass which formed had an infrared spectrum identical with that of authentic octahydrohelvolic acid. No trace of acetic acid could be detected during the pyrolysis.

Attempted preparation of furfurylidene of helvolinic acid. A solution of 111 mg. of helvolic acid, 77 mg. of freshly distilled furfural, 0.4 ml. of 1*N* sodium hydroxide, 4 ml. of water, and 4 ml. of absolute ethanol was allowed to stand in the dark at room temperature for 6 days. The solution was then acidified with cold dilute hydrochloric acid. The solid which formed was collected by suction filtration and crystallization from aqueous ethanol and yielded 65 mg. of crystalline material, m.p. 185–189° dec. The infrared spectrum was identical with that of helvolinic acid and showed no band at 6.23 μ which was present in tetrahydrohelvolinic acid furfurylidene.

Test for a 4,4-dimethyl structure in methyl tetrahydrohelvolate.[26] A solution of 100 mg. of methyl tetrahydrohelvolate and 15.2 mg. of sodium borohydride in 2 ml. of 1% aqueous dioxane was allowed to stand at room temperature for 1 hour. The solution was diluted with water and extracted with ether. The ether solution was dried and the ether was removed on a steam bath. The infrared spectrum of the residue showed strong hydroxyl absorption at 2.90 μ and a carbonyl band at 5.75 μ. No attempt was made to purify this material. The residue was dissolved in 100 ml. of dry hexane and 200 mg. of phosphorus pentachloride was added. This mixture was stirred for 6 hr. with a stream of nitrogen bubbling through the solution.[27] Water was added and after 4 hr. the hexane layer was separated and washed with 5% sodium bicarbonate followed by water. The hexane solution was then dried and evaporated to dryness on a steam bath. The residue was taken up in 30 ml. of purified methylene chloride and treated with ozone at −70° for 1 hr. The mixture was warmed to room temperature and shaken with 100 mg. of zinc dust in 50 ml. of water. The mixture was filtered to remove the zinc dust. The aqueous layer was separated and was treated with 2,4-dinitrophenylhydrazine solution but no acetone-2,4-dinitrophenylhydrazone was isolated on workup.

NMR Spectra.[17] The NMR spectra of the compounds examined are listed below with conditions, solvent and reference compounds used. Concentrations are about 10% in each case. Symbols are listed for the size of each peak as it appeared in the spectrum, l for large, m for medium, and s for small.

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Helvolic acid. 40 Megacycles per second, solvent, deuterochloroform; reference, external chloroform. τ values in ppm. for peaks: 2.48 (s), 3.05 (m), 4.01 (s), 4.25 (s), 4.77 (m), 7.64 (s), 7.90 (l), 8.08 (l), 8.34 (l), 8.39 (l), 8.56 (l), 8.68 (m), 8.83 (l), 9.10 (l).

Methyl helvolate. (See Fig. 1) 40 Megacycles; solvent, deuterochloroform; reference, external methylene chloride. τ values in ppm. for ppm. for peaks: 2.50 (s), 2.74 (s), 4.01 (s), 4.27 (s), 4.77 (m), 6.41 (l), 7.67 (s), 7.93 (l), 8.08 (l), 8.38 (l), 8.43 (l), 8.60 (l), 8.72 (m), 8.87 (l), 9.14 (l).

Methyl tetrahydrohelvolate. 60 Megacycles; solvent, deuterochloroform; reference, internal tetramethyl silane. τ values in ppm. for peaks: 4.09 (s), 4.20 (s), 4.71 (m), 6.33 (l), 7.56 (m), 7.89 (l), 8.02 (l), 8.68 (l), 8.77 (m), 8.88 (m), 9.07 (l), 9.16 (m).

Octahydrohelvolic acid. 60 Megacycles; solvent, deuterochloroform; reference, internal tetramethyl silane. τ values in p.p.m. for peaks: 4.21 (l), 4.72 (m), 7.92 (l), 7.99 (l), 8.45 (m), 8.77 (l), 9.09 (l), 9.19 (l).

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(CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ANDHRA UNIVERSITY)

Chemical Examination of *Embelia ribes*. I. Isolation of a New Constituent, "Vilangin," Its Constitution and Synthesis

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A new constituent, "vilangin" (I) has been isolated from the dry ripe berries of *Embelia ribes*. From a study of its reactions and degradation products, vilangin has been assigned the structure, methylenebis(2,5-dihydroxy-4-undecyl-3,6-benzoquinone). This has been confirmed by synthesis using embelin (XVII) and formaldehyde.

The dry berries of *Embelia ribes* are extensively used in India on account of their anthelmintic and antibiotic properties.[1] The active principle, so far isolated and extensively studied, is embelin.[2] Its constitution and synthesis have also been reported.[2] In all these experiments, the authors used ether, ethyl alcohol, benzene, and ethyl acetate as solvents for extraction. When purified methyl alcohol was used for extraction, besides embelin, a small quantity of a new entity melting at 264–265° was obtained. When methyl alcohol was replaced by purified dioxane, the yield of the new component considerably increased. The new substance is designated by us as "vilangin," a name taken from the vernacular Telugu name Vayuvilanga for *Embelia ribes*. We report in this communication, the isolation, constitution and synthesis of vilangin.

Vilangin is bright orange-yellow in color and is insoluble or sparingly soluble in common organic solvents, but easily soluble in dioxane and nitrobenzene. This low solubility and its occurrence in low yield in the natural berries, contributed largely to the failure of its isolation by earlier workers. It exhibited acidic properties by dissolving in alcoholic sodium, potassium and ammonium hydroxides, forming violet, deep violet, and pale violet solutions from which vilangin was regenerated by acidification. From aqueous alcoholic solutions of the alkalis, however, the corresponding salts were precipitated in crystalline condition. Vilangin also dissolved very slowly in aqueous alcoholic solutions of bicarbonate and carbonate with effervescence. With boric acid in concentrated sulfuric acid solution, it gave an intense green fluorescence under the ultraviolet lamp.

Vilangin gave an analysis corresponding to $C_{35}H_{52}O_8$, a formula confirmed by a Rast molecular weight of 610. It contained no alkoxyl groups, and since it was not cleaved when boiled with hydriodic acid, it was presumed to contain no oxide linkages. Its acidic character and an intense brown color formed with ferric chloride in dioxane indicated the presence of phenolic hydroxyls. On heating with orthophosphoric acid or boiling with concentrated sulfuric acid in dioxane solution, anhydrovilangin (II) was obtained, indicative of two hydroxyl

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