[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF CALIFORNIA, AND THE NAVAL MEDICAL RESEARCH INSTITUTE]

The Lactone Ring of Carpaine

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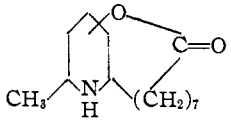
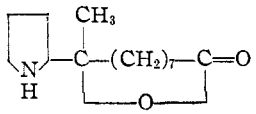
Although carpamic acid is not attacked by periodate, it is oxidized by lead tetraacetate, indicating the presence of a 1,2-aminoalcohol structure. This is confirmed by isolation of 12-ketotetradecanoic acid from the oxidation of nitrogen-free material obtained from degradation of methyl N-methylcarpamate methiodide. The hydroxyl group of carpamic acid is thus at the β'-position III, and carpaine has the structure VI.

In a previous publication,[2] it was shown that the chemistry of carpaine, the papaya alkaloid, was best accommodated by the partial structure I, rather than the structure II which had been originally proposed[3] for this alkaloid. The one structural feature not yet established was the point of attachment of the lactone ring to the piperidine nucleus, and this is the subject of the present report.

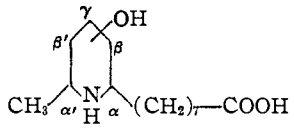


I



II

In considering the various positions in the piperidine nucleus for the terminus of the lactone ring, we first examined the hydrolysis product of carpaine, carpamic acid (III). This substance is an aminoalcohol, and the hydroxyl group must reside



III

at one of the five positions: α, α', β, β' or γ. If it is situated at α or α', carpamic acid should be a carbinolamine (pseudo base), and this possibility was thoroughly investigated. A carbinolamine with acid might be expected to lose the elements of water in forming a salt. Carpamic acid, in contrast, forms normal salts. Also, a carbinolamine might give evidence for the presence of some of the aminocarbonyl form when treated with a suitable carbonyl reagent. Carpamic acid failed to react with 2,4-dinitrophenylhydrazine in acid solution, and with iodine in sodium hydroxide gave no iodoform. Although the evidence thus presented against a carbinolamine structure is negative, it seemed sufficient to eliminate this structure from primary consideration.

The β- and β'-positions were next considered for the hydroxyl group of carpamic acid. If either were the case, then carpamic acid should be a 1,2-aminoalcohol and should be subject to cleavage by periodate or lead tetraacetate.[4] When carpamic acid at 25° and *p*H 8 was treated with a periodate solution, no oxidation occurred. Since it has been shown[4] that periodate oxidation of amino alcohols probably involves the free amine, and since carpamic acid, in which the nitrogen has a *p*Ka of 10.4[2], exists only to the extent of 0.4% as the free base at *p*H 8, this oxidation was repeated at *p*H 8.9 and 10.2. Again no measurable oxidation was observed. At higher temperatures oxidation did take place, but it was indiscriminate and of no structural significance.

Carpamic acid was then treated with lead tetraacetate and was found to undergo oxidation at 25°. One mole of oxidant was smoothly consumed in 30 hours, after which consumption continued but at a slightly slower rate, a behavior frequently encountered with aminoalcohols.[4] In attempting to isolate acetaldehyde from the reaction mixture, volatile aldehydic material was obtained, but since a similar behavior was observed from a solution containing only lead tetraacetate, this attempt was abandoned.

A possible rationale for the resistance of carpamic acid to periodate oxidation may be found in the mechanism suggested[5] for the oxidation of glycols. If a similar cyclic ester of periodic acid is involved in the case of 1,2-aminoalcohols, the strongly alkaline solutions needed in order to maintain an appreciable fraction of carpamic acid as the free base may prevent formation of this intermediate ester. Thus with strongly basic aminoalcohols, the *p*H needed for intermediate ester formation and the *p*H needed for existence as the free base, both necessary conditions for oxidation to occur, may be mutually exclusive in some cases.

Accepting oxidation of carpamic acid by lead tetracetate as evidence for the hydroxyl group being at β or β', definitive evidence was then sought in degradation to an oxygenated myristic acid. Since the alcoholic oxygen function had been lost during the course of previous degradative studies[6] starting with material having an intact lactone ring, degradation of a carpamic acid derivative in which the lactone no longer exists and the alcoholic hydroxyl is retained was considered more likely to yield material with the desired characteristics.

Such a derivative was found in methyl N-methylcarpamate methiodide (IV) which was prepared in excellent yield from carpaine by heating with methyl iodide in methanol over anhydrous potassium carbonate. Methoxyl analysis, the presence of a strong hydroxyl band (3.0 μ) in the infrared,

(1) Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md. The opinions contained herein are the writers' and are not necessarily those of the Navy Department.

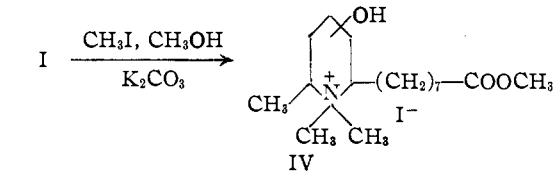
(2) H. Rapoport and H. D. Baldridge, Jr., THIS JOURNAL, **74**, 5365 (1952).

(3) G. Barger, A. Girardet and R. Robinson, *Helv. Chim. Acta*, **16**, 90 (1933); G. Barger, R. Robinson and T. S. Work, *J. Chem. Soc.*, 711 (1937).

(4) G. E. McCasland and D. A. Smith, THIS JOURNAL, **73**, 5164 (1951).

(5) C. C. Price and M. Knell, *ibid.*, **64**, 552 (1942).

(6) H. Rapoport and H. D. Baldridge, Jr., *ibid.*, **73**, 343 (1951).

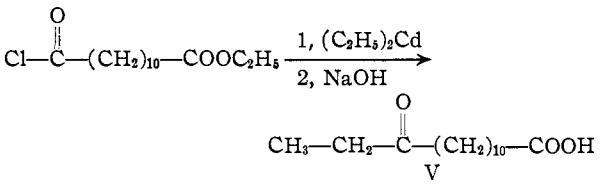


IV

and failure to liberate any free amine on making its aqueous solution alkaline establish IV as its structure. By converting this compound to methocarbonate, dry distilling, hydrogenating and then repeating this process through many cycles, saturated nitrogen-free material was finally obtained.

Oxidation of this nitrogen-free material with chromic oxide in glacial acetic acid yielded a dibasic acid, m.p. 125–126°, equivalent weight 116, and a keto acid, m.p. 81.3–81.9°. The dibasic acid was identified by mixed melting point determinations as dodecandioic acid (m.p. 126–127°, equivalent weight 115) from which it follows that the hydroxyl group probably had been attached to either the twelfth or thirteenth carbon. The keto acid isolated would then very likely be either 12- or 13-ketotetradecanoic acid. The latter compound is known,[7] and has been reported to melt at 75°. Furthermore, it would be expected to give iodoform on hypoiodite oxidation.

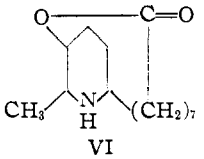
Since the degradation keto acid yielded no iodoform when treated with iodine in alkali and melted appreciably higher than the reported 13-keto acid, 12-ketotetradecanoic acid (V) was synthesized for comparison with the degradation acid from the ethyl ester acid chloride of dodecandioic acid and diethylcadmium.



V

This synthetic keto acid was found to melt at 81.0–81.6°, and this melting point was not lowered on admixture with the degradation keto acid. A similar result was obtained with the semicarbazones of the two acids.

The isolation of 12-ketotetradecanoic acid from the oxidation of the nitrogen-free degradation material is consistent with the oxidation of carpamic acid by lead tetraacetate and proves that the hydroxyl group of carpamic acid (III) is at the β'-position. The structure of carpaine thus has been established as VI.



VI

Experimental[8]

Periodate Oxidation of Carpamic Acid (III).—Using the standard procedures,[9] carpamic acid[2] was subjected to the action of periodate in solutions buffered at *p*H 8, 8.9 and 10.2. At 25°, no discernible oxidation occurred during a 24-hour period, and when the temperature was raised to 40° and above, oxidation was rapid and indiscriminate with no apparent change in rate after the consumption of one mole of oxidant. No identifiable material could be isolated from these higher-temperature oxidation experiments.

Lead Tetraacetate Oxidation of Carpamic Acid.—Carpamic acid in absolute acetic acid was treated with lead tetraacetate[10] according to the standard oxidation conditions[11] at 25°. After 30 hours, one mole of oxidant had been consumed per mole of carpamic acid, and after 100 hours, two moles had been consumed.

Methyl N-Methylcarpamate Methiodide (IV).—A solution of 5.02 g. (0.021 mole) of carpaine[12] in a mixture of 44 g. (0.31 mole) of methyl iodide and 250 ml. of absolute methanol was heated under reflux with 20 g. of anhydrous potassium carbonate for 60 hours. After 12 hours of the heating period had elapsed, a mixture of 40 g. of methyl iodide and 20 g. of anhydrous potassium carbonate was added, and heating was resumed. The addition of methyl iodide (40 g.) was repeated after 24 additional hours of refluxing, which was then continued for 24 hours. The hot liquid phase was decanted through a suction filter, and the residue was digested on the steam-bath with several portions of absolute methanol, which were also decanted through the filter. After the solvent had been removed on the steam-bath using an air stream, the dry residue was digested several times under reflux with dry chloroform. Evaporation of the filtered chloroform solutions gave an almost colorless glass which solidified as a froth in the vacuum desiccator. Crystallization was effected by adding pentane slowly to a cold absolute ethanolic solution and resulted in 8.1 g. (90% yield) of material melting at 113–114°, $[\alpha]^{21}D$ −13.5° (*c* 0.93, ethanol).

Anal. Calcd. for $C_{17}H_{34}O_3NI$: C, 47.8; H, 8.0; OCH_3, 7.3. Found: C, 47.7; H, 8.1; OCH_3, 7.3.

Degradation of Methyl N-Methylcarpamate Methiodide to Nitrogen-free Material.—Degradation of 8 g. (0.019 mole) of methyl N-methylcarpamate methiodide was accomplished essentially as described previously.[6] Using the above methylation procedure to remethylate basic material and recycling a total of six times, a 29% yield of nitrogen-free material was obtained, calculated as the saturated, hydroxy methyl ester.

Oxidation of Nitrogen-free Material.—A 485-mg. sample of nitrogen-free material, dissolved in 9 ml. of glacial acetic acid, was mixed at room temperature with 6 ml. of a 5% solution of chromium trioxide in glacial acetic acid. After standing at room temperature one hour, adding 25 ml. of water, and bubbling in sulfur dioxide for one minute, the solution was extracted thoroughly with ether. Evaporation of the combined ether extracts after several washes with water and 10% potassium hydroxide solution, left 300 mg. of neutral residue.

The combined water and 10% potassium hydroxide washes of this ether extract were acidified to congo red, extracted with ether, and the ether extract was first washed with dilute potassium dichromate solution to remove sulfur dioxide, then with water and finally dried. The oily residue 100 mg.) realized on evaporation of the ether was crystallized once from water and twice from benzene to give an acid, m.p. 125–126°, equiv. wt., 116. The melting point of an authentic sample[13] of dodecandioic acid (m.p. 126–127°, equiv. wt., 115) was not depressed on admixture with this degradation acid (mixed m.p. 125.4–126.8°).

The neutral fraction (300 mg.) isolated above was hydrolyzed in a mixture of 15 ml. of glacial acetic acid and 15 ml. of 6 *N* sulfuric acid by heating under reflux overnight. After cooling and diluting with 40 ml. of saturated ammonium sulfate solution, the solution was extracted with

(7) M. Stoll and A. Commarmont, *Helv. Chim. Acta*, **31**, 1435 (1948); D. C. Grimshaw, J. B. Guy and J. C. Smith, *J. Chem. Soc.*, 68 (1940); G. M. Robinson, *ibid.*, 1543 (1934).

(8) All melting points are corrected; microanalyses were performed by the Microchemical Laboratory, University of California.

(9) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 357.

(10) W. S. McClenahan and R. C. Hockett, THIS JOURNAL, **60**, 2061 (1938).

(11) R. C. Hockett, M. T. Dienes and H. E. Ramsden, *ibid.*, **65**, 1474 (1943).

(12) We are again indebted to Dr. Bywater and the S. B. Penick Company for securing the papaya leaves and carrying out the initial stages of the extraction (see ref. 6).

(13) This sample was very generously supplied by Dr. J. Cason.

ether. The ether solution was then extracted with 1 *N* potassium carbonate solution and this in turn was acidified to congo red and extracted with ether. Crystallization of the residue (250 mg.), after evaporating the ether, from petroleum ether and hexane gave 30 mg. of material, m.p. 81.3–81.9°.

Anal. Calcd. for $C_{14}H_{26}O_3$: C, 69.4; H, 10.8. Found: C, 69.1; H, 11.2.

The mixed melting point of this degradation keto acid with an authentic sample of 12-ketotetradecanoic acid (m.p. 81–81.6°), prepared below, was 81.3–82°.

Evaporation of the petroleum ether–hexane mother liquors from crystallization of the degradation keto acid left a residue which was subjected to further crystallization, fractional sublimation, counter-current extraction[14] and chromatography,[15] all of which failed to yield pure material. However, use of Girard reagent T[16] gave a ketonic fraction which was converted to its semicarbazone, m.p. 140–141° after several crystallizations from ethanol. An authentic sample of 12-ketotetradecanoic acid formed a semicarbazone, m.p. 142–143°, and a mixture with the degradation keto acid semicarbazone melted at 142–143°.

Ethyl 12-Ketotetradecanoate.—According to the general procedure of Cason,[17] a solution of 13.1 g. (0.047 mole) of the ethyl ester acid chloride of dodecandioic acid in 40 ml. of dry benzene[13] was rapidly added (over a period of about 10 minutes) with vigorous stirring to a solution of diethylcadmium [freshly prepared from 0.1 mole of ethylmagnesium bromide and 10 g. (0.055 mole) of anhydrous cadmium chloride] in 75 ml. of dry benzene, and the reaction mixture was heated under reflux for 15 minutes followed by cooling in an ice-bath. After adding a mixture of 80 g. of ice and 30 ml. of 6 *N* sulfuric acid to the reaction mixture, the keto ester was extracted into benzene, which was then washed water and 0.5 *M* potassium carbonate, rewashed with water, and dried over anhydrous magnesium sulfate. Evaporation of the benzene and fractionation through a one-meter Podbielniak column gave 7.3 g. (58%) of pure ethyl 12-ketotetradecanoate, b.p. 149–150° (1.5 mm.), m.p. 34.5–35.8°.

Anal. Calcd. for $C_{16}H_{30}O_3$: C, 71.1; H, 11.2. Found: C, 70.9; H, 11.1.

The semicarbazone was prepared in the usual manner, recrystallized three times from aqueous ethanol and dried at 56° *in vacuo*, m.p. 86.5–88.0°.

Anal. Calcd. for $C_{17}H_{33}O_3N_3$: C, 62.3; H, 10.2; N, 12.8. Found: C, 62.0; H, 10.1; N, 12.9.

12-Ketotetradecanoic Acid (V).—A 5.06-g. (18.7 millimoles) sample of ethyl 12-ketotetradecanoate was dissolved in a mixture of 50 ml. of 95% ethanol and 25 ml. of 2 *N* aqueous sodium hydroxide and heated under reflux on the steam-bath for 3.5 hours. The mixture was evaporated to dryness on the steam-bath under a stream of air, the residue was dissolved in warm water and the solution was filtered, acidified and cooled. Recrystallization of the precipitate from a mixture of petroleum ether and heptane gave 3.77 g. (83%) of keto acid, m.p. 80–81°, which was recrystallized three times from hexane to give pure 12-ketotetradecanoic acid, m.p. 81–81.6°.

Anal. Calcd. for $C_{14}H_{26}O_3$: C, 69.4; H, 10.8. Found: C, 69.2; H, 10.8.

The semicarbazone prepared in the usual manner, recrystallized three times from aqueous ethanol and twice from 95% ethanol, and dried at 100° (1 mm.), melted at 142–143°.

Anal. Calcd. for $C_{15}H_{29}O_3N_3$: C, 60.2; H, 9.8; N, 14.0. Found: C, 60.2; H, 9.8; N, 14.1.

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(14) J. Fugger, K. T. Zilch, J. A. Cannon and H. J. Dutton, THIS JOURNAL, **73**, 2861 (1951).

(15) C. S. Marvel and R. D. Rands, Jr., *ibid.*, **72**, 2642 (1950).

(16) A. Girard and G. Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936).

(17) J. Cason, THIS JOURNAL, **68**, 2078 (1946).

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, SMITH, KLINE AND FRENCH LABORATORIES]

Colchicine. Derivatives of Trimethylcolchicinic Acid[1,2]

BY ROBERT F. RAFFAUF, ANN L. FARREN AND GLENN E. ULLYOT

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Previous work on the chemistry of ring C in colchicine and its derivatives has been extended to include derivatives of trimethylcolchicinic acid, for which the existence of isomeric compounds analogous to the normal and iso forms obtained from colchiceine has been established. Several of these compounds are described for the first time and their configurations have been determined by relationship, through chemical and physical methods, to the parent compounds colchicine and isocolchicine. An improved preparation of trimethylcolchicinic acid is described.

For the preparation of compounds of interest to a comprehensive study of the effect of structural variation on the biological activity of colchicine derivatives we required large amounts of trimethylcolchicinic acid (II). This compound had been prepared by Zeisel[3] by neutralization of the hydrochloride obtained by acid hydrolysis of colchicine (I), and later by Windaus[4] who isolated it in the form of its dihydrochloride. In our hands these earlier methods left much to be desired from a preparative standpoint, although recently Šantavý[5] reported yields of 60–76% using Zeisel's method. By substitution of 20–30% sulfuric acid for hydrochloric acid we have obtained an easily isolable product in 80% yield.

The chemical behavior of trimethylcolchicinic acid (desacetylcolchiceine) was anticipated, in part, on the basis of the currently accepted tropolone formulation of ring C in colchiceine. Thus, methylation gave a mixture of methyl ethers IIIa, IIIb which was separated either chromatographically or by fractional crystallization of the *d*-tartrates IVa, IVb. Šantavý[5] reported that he was unable to separate either the free ethers or their salts. The configurations of our isomers were established by acetylation to give in one case IIIb colchicine and in the other IIIa isocolchicine. The amides Va, Vb were prepared from the ethers by reaction with ammonia.

The isomeric derivatives of colchiceine exhibit distinct differences in their physical properties.[6] Data for the derivatives of trimethylcolchicinic acid (Table I) are in agreement with this previous experience.

(1) This investigation was supported (in part) by a research grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

(2) Paper presented (in part) before the Fifth Meeting-in-Miniature of the Philadelphia Section of the American Chemical Society, January 29, 1953.

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(6) R. M. Horowitz and G. E. Ullyot, THIS JOURNAL, **74**, 587 (1952).