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.

BERKELEY, CALIFORNIA

(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

RECEIVED MAY 19, 1953

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.

(3) S. Zeisel, *Monatsh.*, **9**, 1–30 (1888).

(4) A. Windaus, *Sitzber. Heidelberg Akad. Wiss., Math. Naturw. Klasse*, 1–27, 2. Abh. (1911).

(5) F. Šantavý, *Chem. Listy*, **46**, 280 (1952).

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

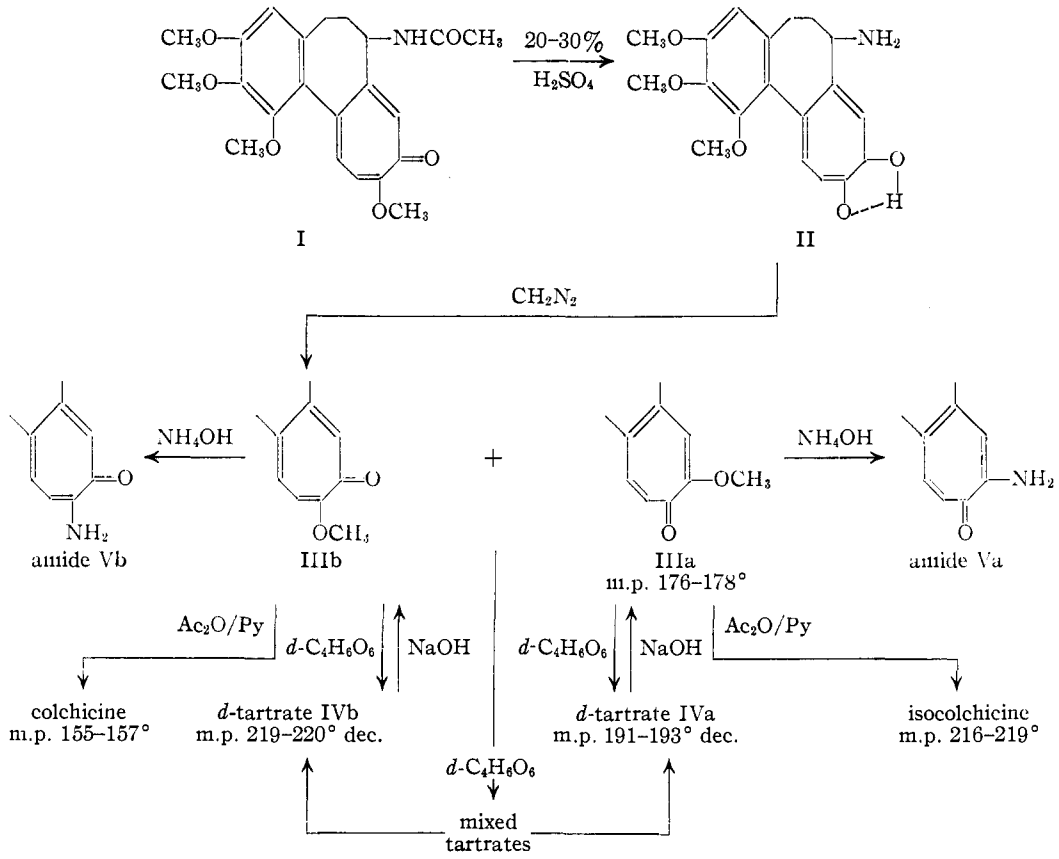

TABLE I

Compound	M.p., °C.	$[\alpha]^{25}D$ (c, chl.)	Ultraviolet λ_{max} (log ε)	Infrared 7 μ region
Trimethylcolchicinic acid methyl ether	Amorph.	−159° (0.653)	246 (4.40) 351 (4.22)	Double band
Isotrimethylcolchicinic acid methyl ether	176–178	−240 (1)	245 (4.50) 343 (4.29)	Single band
Trimethylcolchicinic acid amide	Amorph.	−175 (0.785)	245.5 (4.49) 355 (4.31)	
Isotrimethylcolchicinic acid amide	112–125 226–228	−236 (0.93)	245 (4.42) 354 (4.27)	

Further confirmation of the structure of the amorphous amide Vb was obtained by conversion to the N-acetylcolchiceine amide of Šantavý.[5] The anti-mitotic activity of these derivatives is being studied and will be reported elsewhere.[7]

Experimental

Trimethylcolchicinic Acid (II).—A mixture of 53 g. of purified colchicine,[8] 250 cc. of concentrated sulfuric acid and 1 l. of water was heated on the steam-bath with stirring for 5 hours. The hot solution was neutralized (*p*H 7–7.5) with solid soldium carbonate and allowed to cool to room temperature. The light yellow frothy mass was filtered, and the collected solid was washed with cold water and sucked dry. The product was crystallized from ethanol.* The air-dried, light yellow needles, m.p. 152–158°, weighed 35 g.; 1–2 g. of additional material was obtained by extraction of the aqueous filtrates with chloroform.

The product retained solvent tenaciously and on drying deepened in color. For analysis, a sample was thrice crystallized from ethanol and dried for 10 hours *in vacuo* at 100°; m.p. 155–157°.

Anal. Calcd. for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.16; OCH_3, 27.08. Found: C, 65.92; H, 6.11; OCH_3, 26.87; $[\alpha]^{25}D$ −184.5° (*c* 1 in chloroform).

Trimethylcolchicinic Acid Methyl Ethers (III).—Twenty grams of crude II (containing alcohol of crystallization) was dissolved in 400 cc. of methylene chloride and treated with a methylene chloride–ether solution (500 cc., 1:1) of diazomethane prepared in the usual way[9] from 13 g. of nitrosomethylurea and 60 cc. of 40% potassium hydroxide. The mixture was kept at 0–5° until all of the solid had dissolved and the solution no longer gave a green color with 1% ferric chloride solution. A small amount of finely divided amorphous material was removed by filtration, and the solvents were evaporated *in vacuo*. The crude product was dissolved in chloroform, the chloroform solution was washed with dilute sodium hydroxide and water and was evaporated to dryness *in vacuo*. The bright yellow amorphous residue weighed 21.5 g. It was redissolved in pure chloroform[10] and put on a 4 × 22 cm. column of neutral alumina prepared in the same solvent.

(a) The fraction obtained by elution with pure chloroform (1500 cc.) gave, on evaporation, 11.5 g. of bright yellow amorphous material which crystallized from alcohol–ether to yield a light yellow solid, m.p. 174–176°. Three additional crystallizations from alcohol–ether gave light yellow prisms of isotrimethylcolchicinic acid methyl ether, m.p. 176–178°. For analysis a sample was dried for 5 hours *in vacuo* at 100°.

Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49. Found: C, 67.21; H, 6.70; $[\alpha]^{25}D$ (*c* 1 in chloroform), −240°; λ_{max} (log ε), 245 mμ (4.50), 343 mμ (4.29) (*c* 4.11 × 10^{-5} *M* in 95% ethanol).

(7) For the effect of trimethylcolchicinic acid methyl ether *d*-tartrate *vs.* mouse Sarcoma 37 see J. Leiter, V. Downing, J. L. Hartwell and M. J. Shear, *J. Nat. Cancer Inst.*, **13**, 379 (1952).

(8) R. M. Horowitz and G. E. Ullyot, *Science*, **115**, 216 (1952).

* NOTE ADDED IN PROOF: Industrial alcohol, formula No. 30 is intended. Subsequent to the submission of this manuscript we found that pure ethanol was unsuitable for the crystallization of trimethylcolchicinic acid. Addition of a small amount of methanol, however, resulted in rapid, satisfactory crystallization.

(9) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

(10) Because the chromatographic separation of many isomeric colchicine derivatives depends upon small differences in the amount of alcohol in chloroform–methanol mixtures we have made it a practice to remove the preservative (ethanol) from commercial chloroform before use.

The infrared spectrum (Nujol mull) exhibited a single band at *ca.* 7 μ previously shown to be characteristic of the iso configuration.[7] A 100-mg. sample, after heating for 15 minutes on the water-bath with 1 cc. of acetic anhydride and 1.5 cc. of dry pyridine and removal of the solvents *in vacuo* gave isocolchicine, light tan prisms from ethyl acetate–ether, m.p. 216–219° alone and when mixed with an authentic specimen. A 100-mg. sample was treated with *d*-tartaric acid in boiling acetone. A light yellow micro-crystalline powder was obtained which, after digestion in hot alcohol–acetone (1:1) and cooling was removed by filtration and dried. It decomposed at 190–191°.

(b) The remainder of the product (9.97 g.) was eluted from the chromatograph column with methanol (750 cc.). The solvent was removed *in vacuo*, the residue was redissolved in pure chloroform and rechromatographed on a 4 × 12.5 cm. column of neutral alumina. That portion (4.1 g.) eluted by chloroform (780 cc.) and chloroform–0.5% methanol (140 cc.) was identical with the iso-ether obtained in (a); the total weight of iso-ether was thus 15.6 g. The fraction eluted by chloroform–1% and chloroform–2% methanol weighed 4.9 g. This *n*-trimethylcolchicinic acid methyl ether could not be crystallized from a variety of solvents. A sample acetylated as described above gave colchicine, colorless needles from ethyl acetate–ether; m.p. 155–157° alone and when mixed with an authentic sample. The infrared spectrum (Nujol mull) of the amorphous product exhibited a double band at *ca.* 7 μ characteristic of the normal configuration.[7] This was further confirmed by the ultraviolet spectrum, λ_{max} (log ϵ), 246 mμ (4.40), 351 mμ (4.22), *c* 7.3 × 10^{-5} *M* in 95% ethanol, and the specific rotation, $[\alpha]^{25}D$ −159° (*c* 0.6528 in chloroform). A sample was treated with *d*-tartaric acid in boiling alcohol. The precipitate which formed was recrystallized from alcohol–water, colorless needles, m.p. 219–221° dec.

Trimethylcolchicinic Acid Methyl Ether *d*-Tartrates (IV).—A sample of the crude mixed ethers prepared as described above was dissolved in ethanol, *d*-tartaric acid (10% excess) was added, and the mixture was heated on the hot-plate until a solid began to separate. After cooling overnight the mixture was filtered and the solid was resuspended in hot ethanol. Water was added dropwise until solution was effected, the solution was treated with Norit, filtered and again allowed to cool. The white micro-crystalline powder thus obtained was then crystallized from acetone–water and dried over phosphorus pentoxide; m.p. 218.5–220° dec. and identical with the sample prepared from IIIb.

Anal. Calcd. for $C_{24}H_{29}NO_{11}\cdot H_2O$: C, 54.85; H, 5.95. Found: C, 54.79, 54.53; H, 5.36, 5.63.

Water of crystallization was lost only after protracted drying in high vacuum at 100°.

Anal. Calcd. for $C_{24}H_{29}NO_{11}$: C, 56.80; H, 5.76. Found: C, 56.80; H, 6.38.

The filtrates from the *n*-tartrate were evaporated to dryness *in vacuo*. The residue was triturated with hot acetone to give a yellow micro-crystalline powder, m.p. 181–184° dec. The decomposition point was raised to 191–193° after two digestions with alcohol–acetone (1:1). The product was identical to that obtained from IIIa above. A final crystallization from methanol gave fine light yellow needles which turned to a glass between 145–155° and finally decomposed at 177–178°.

Anal. Calcd. for $C_{24}H_{29}NO_{11}\cdot CH_3OH$: C, 55.65; H, 6.17. Found: C, 55.78; H, 6.16.

After grinding the sample and drying to constant weight in high vacuum at 110°, the methanol of crystallization was lost.

Anal. Calcd. for $C_{24}H_{29}NO_{11}$: C, 56.80; H, 5.76; OCH_3, 24.44. Found: C, 56.98; H, 6.02; OCH_3, 24.47, 24.51.

The ratio of *n*- to isotrimethylcolchicinic acid methyl ether obtained by this procedure was approximately 1:3.

Triethylcolchicinic Acid Amides (V).—A 5.07-g. sample of isotrimethylcolchicinic acid methyl ether *d*-tartrate was dissolved in 200 cc. of concentrated ammonium hydroxide and the mixture was allowed to stand in a stoppered flask for 24 hours at room temperature. The solution was filtered to remove a small amount of gummy material and was then evaporated *in vacuo*. After all of the ammonia had been removed the bright yellow solid which had separated was removed by filtration, washed with cold water and dried (1.2 g.). After drying to constant weight *in vacuo* at 80° the product melted from 112–125°, resolidified between 128–133° and remelted at 226–228°.

Anal. Calcd. for $C_{19}H_{22}N_2O_4$: C, 66.55; H, 6.48; N, 8.18. Found: C, 66.66; H, 6.69; N, 8.10.

The ultraviolet spectrum was taken in 95% ethanol (*c* 5.22 × 10^{-5} *M*), λ_{max} (log ϵ), 245 mμ (4.42), 354 mμ (4.27); $[\alpha]^{25}D$ −236° (*c* 0.93 in chloroform).

Three grams of trimethylcolchicinic acid methyl ether *d*-tartrate was treated in a similar fashion. The aqueous ammoniacal solution in this case did not deposit a solid product on evaporation of the ammonia. The solution was therefore adjusted to *p*H 7 and extracted with chloroform. The chloroform layer was washed with cold water and evaporated to dryness leaving 730 mg. of a yellow amorphous residue. This could not be induced to crystallize from a variety of solvents. After drying to constant weight *in vacuo* at 80° it showed $[\alpha]^{25}D$ −175° (*c* 0.785 in chloroform), λ_{max} (log ϵ), 245.5 mμ (4.49), 355 mμ (4.31), *c* 4.58 × 10^{-5} *M* in 95% ethanol.

A solid derivative was prepared by acetylation in acetic anhydride–pyridine. The crude product was purified by passage through a small column of neutral alumina (eluant, chloroform–1% methanol) and crystallized from ethyl acetate–ether then from methanol–ether. **N-Acetylcolchiceine amide**[5] was obtained, m.p. and mixed m.p. with a sample prepared by acetylation of colchiceine amide, 152–154° with softening at *ca.* 145°. The compound was dried *in vacuo* at 80° prior to analysis.

Anal. Calcd. for $C_{23}H_{26}N_2O_6$: C, 64.77; H, 6.15. Found:[11] C, 64.76; H, 6.49.

Acknowledgment.—The ultraviolet spectra were determined using a Cary recording spectrophotometer, model 11M and the infrared spectra were taken on a Perkin–Elmer infrared spectrometer, model 12C by Mr. S. Rump; microanalyses were done by Mrs. O. Preis and Miss D. Aitken of these laboratories whose coöperation is greatly appreciated.

PHILADELPHIA, PENNA.

(11) These values are corrected for a small amount (0.73%) of ash which we believe to be due to the formation of stable metal complexes by many of the colchiceine amides. The inorganic matter may be introduced by ammonia and/or its action on glass during the course of the reaction.