Anal. Calcd. for $C_{16}H_{10}N_2O_3$: C, 69.06; H, 3.62. Found: C, 69.12; H, 3.69.

1-(4-Pyridylmethyl)-6,7-methylenedioxyisoquinoline (VI).—A solution of 0.35 g. of 1-isonicotinyl-6,7-methylenedioxyisoquinoline (IX) in 5 cc. of glacial acetic acid was stirred on the steam-bath while 1.77 g. of zinc dust was added over a period of three hours. Acetic acid was added from time to time to keep the volume of the solution constant. At the end of three hours the mixture was filtered and the residue washed with 20 cc. of boiling water. After removal of zinc with hydrogen sulfide and washing the precipitate with hot water, the filtrate was concentrated on the steam-bath to 20 cc., neutralized with sodium carbonate, and an excess of aqueous ammonia added. The free base was extracted with six 10-cc. portions of chloroform, and the extract dried over sodium sulfate and evaporated to dryness. The residue was crystallized from petroleum solvent (b.p. 55–85°) to give 0.04 g. of white crystals, m.p. 167–169°. After three more crystallizations and drying at 80° and 20 mm., it melted at 171–173°.

Anal. Calcd. for $C_{16}H_{12}N_2O_2$: C, 72.71; H, 4.58. Found: C, 72.31; H, 4.61.

Absorption Spectra.—All of the absorption spectra measurements were made on freshly prepared solutions in 95% alcohol that had been distilled from potassium hydroxide. The concentrations were approximately 4×10^{-5} molar. A Beckman spectrophotometer, model DU, was used.

STANFORD, CALIF.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

Mycomycin. II. The Structure of Isomycomycin, an Alkali-Isomerization Product of Mycomycin[1,2]

BY WALTER D. CELMER AND I. A. SOLOMONS

RECEIVED FEBRUARY 1, 1952

Isomycomycin, a crystalline alkali-isomerization product of the antibiotic mycomycin, has been prepared and characterized. The structure of isomycomycin has been deduced from chemical and physical evidence as 3,5-tridecadiene-7,9,11-triynoic acid, $CH_3C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}COOH$.

The antibiotic mycomycin, an elaboration product of *Norcardia acidophilus*,[3] has been previously isolated in crystalline form and characterized.[4] This unstable compound was shown to be an optically active, highly unsaturated carboxylic acid having the empirical formula $C_{13}H_{10}O_2$ and structure[1] 3,5,7,8-tridecatetraene-10,12-diynoic acid (I).

$$HC{\equiv}C{-}C{\equiv}C{-}CH{=}C{=}CH{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CO_2H$$

I

Mycomycin undergoes extensive rearrangement in normal aqueous alkali metal hydroxide solution at 27° involving an allene to acetylene isomerization accompanied by migration of existing acetylenic bonds. The rearranged acid, isomycomycin, has been assigned[1] the structure 3,5-tridecadiene-7,9,11-triynoic acid (II).

$$CH_3{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CO_2H$$

II

It is the purpose of this paper to describe further the preparation and derived structure of isomycomycin.

The extreme instability of mycomycin at the *p*H of its alkali metal salts and in liquid ammonia[4] prompted further investigation of possible base-catalyzed rearrangement reactions. Attempts to isomerize mycomycin in alcoholic alkali[5] and in various amines invariably gave black tarry products. However, when an excess of aqueous potassium, sodium or lithium hydroxide is added to mycomycin, the crystalline salt of an isomeric acid separates from solution within a few minutes. Acidification of a dilute aqueous solution of this salt precipitates the free acid, designated "isomycomycin," which can be recrystallized from ether–hexane. Isomycomycin is about one-fourth as active *in vitro* as mycomycin against *Mycobacterium tuberculosis* H37Rv, however, the activity against the mycomycin assay organism, *B. subtilis*,[4] is extremely low. Like mycomycin, isomycomycin has the empirical formula $C_{13}H_{10}O_2$ and takes up eight moles of hydrogen upon catalytic hydrogenation, yielding *n*-tridecanoic acid, thus excluding the possibility of branching or ring structure in the original molecule and establishing the length of the carbon chain. However, unlike mycomycin, the isomeric compound is optically inactive and analyzes for one C-methyl group (mycomycin has none). Its infrared spectrum exhibits no allenic nor terminal acetylenic absorption. Isomycomycin reacts with diazomethane to give a crystalline methyl ester, which in contrast to mycomycin methyl ester, does not react with alcoholic silver nitrate, substantiating the absence of a $-C{\equiv}CH$ functional group.[6] The ester reacts smoothly with fused maleic anhydride at 70°, yielding a crystalline monoaddition product.

The infrared spectra (Fig. 1) of isomycomycin, its methyl ester and the maleic anhydride adduct of the methyl ester exhibit a strong absorption band near 2200 cm.$^{-1}$ which is interpreted as a disubstituted carbon–carbon triple bond stretching frequency.[7] It has been observed that compounds containing conjugated polyacetylenic linkages have well-defined ultraviolet absorption, with the long wave length maxima spacings (1900–2300 cm.$^{-1}$)

(1) First reported in a Communication to the Editor, THIS JOURNAL, 74, 1870 (1952).

(2) Presented before the Division of Organic Chemistry at the Milwaukee meeting of the American Chemical Society, April 2, 1952.

(3) E. A. Johnson and K. L. Burdon, *J. Bact.*, **54**, 281 (1947).

(4) W. D. Celmer and I. A. Solomons, Abstracts 121st ACS Meeting, Milwaukee, March–April, 1952; THIS JOURNAL, **74**, 2245 (1952).

(5) (a) T. Moore, *Biochem. J.*, **31**, 138 (1937); (b) J. P. Kass and G. C. Burt, THIS JOURNAL, **61**, 3292 (1939).

(6) A. Behal, *Ann. chim.*, **15**, 408 (1888).

(7) (a) H. W. Thompson, *J. Chem. Soc.*, 328 (1948); (b) J. H. Wotiz and F. A. Miller, THIS JOURNAL, **71**, 3441 (1949); (c) N. B. Colthup, *J. Optical Soc. Am.*, **40**, 397 (1950).

TABLE I

LIGHT ABSORPTION DATA

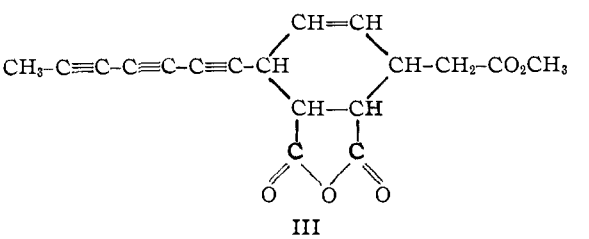
Compound		1st series			2nd series			
		A						
Isomycomycin methyl ester[a]	λ_{max}^{mu}	246 (infl.)	257.5	267	287.5	305.5	324	347
	$\epsilon \times 10^{-3}$	23	58	110	13	27	42	34
	$\Delta\nu'$ (cm.$^{-1}$)					2100	1900	2100
2,10-Dodecadiene-4,6,8-triyne[b]	λ_{max}^{mu}	245	259	269	289	305.5	325	348
	$\epsilon \times 10^{-3}$	25.4	43.1	76.4	11.4	18.4	25.8	20.1
	$\Delta\nu'$ (cm.$^{-1}$)					1900	2000	2000
Dehydromatricaria ester[c]	λ_{max}^{mu}	244.5	256.0		286.7	303.7	324.0	348.6
	$\epsilon \times 10^{-3}$	41	49		6	13	20	17
	$\Delta\nu'$ (cm.$^{-1}$)					2000	2100	2100
		B						
Maleic anhydride adduct of isomycomycin methyl ester[d]	λ_{max}^{mu}	208 (infl.)	213			272.5	289	310
	$\epsilon \times 10^{-3}$	82	110			0.45	0.43	0.17
	$\Delta\nu'$ (cm.$^{-1}$)						2000	2300
1,6-Bis-(1-hydroxy-cyclohexyl)-1,3,5-hexatriyne[e]	λ_{max}^{mu}	208	217		260	275	290	312
	$\epsilon \times 10^{-3}$	87	119		0.19	0.21	0.24	0.16
	$\Delta\nu'$ (cm.$^{-1}$)					2100	1900	2400

[a] Diethyl ether solution. [b] Solvent unspecified, ref. 8c. [c] Hexane solution, ϵ values estimated from spectrum in ref. 16. [d] Methanol solution. [e] Methanol solution, ϵ values estimated from spectrum in ref. 10c.

corresponding to acetylenic vibrational frequencies.[8,9] This property of conjugated polyacetylenic compounds is also observed in the ultraviolet spectrum of isomycomycin as well as its methyl ester and the maleic anhydride adduct of the ester (see Table I and Figs. 2 and 3).

The ultraviolet absorption spectrum of the maleic anhydride adduct of isomycomycin methyl ester is characterized by low intensity of the maxima at the longer wave lengths and high intensity of the maxima at shorter wave lengths (Table I, B and Fig. 2). This type of light absorption has been previously associated with compounds containing conjugated di- and triacetylene linkages as the principal chromophores.[10] The positions of the maxima agree closely with those described for dimethyltriacetylene[11] and 1,6-bis-(1-hydroxycyclohexyl)-1,3,5-hexatriyne[10c] (Fig. 2). The above-mentioned light absorption properties are compatible with structure III for the maleic anhydride adduct of isomycomycin methyl ester.

In Fig. 1A, the presence of ethylenic bonds in isomycomycin is inferred from the strong infrared absorption in the hydrogen bending region near 990



III

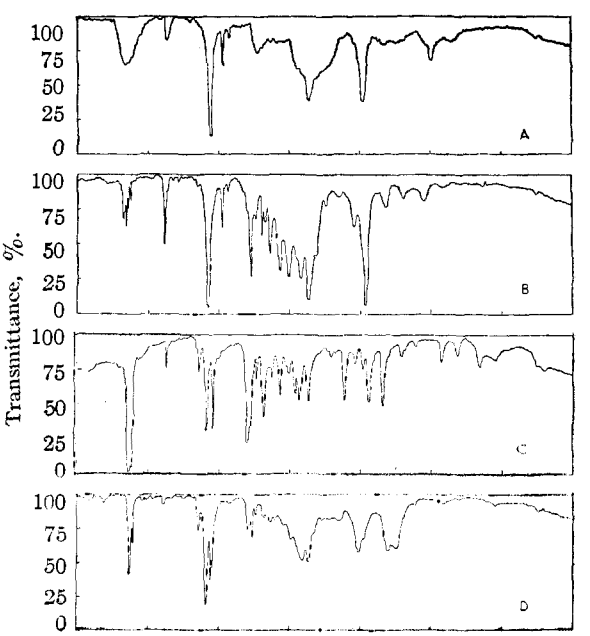


Fig. 1.—Infrared spectra: A, isomycomycin (dioxane); B, isomycomycin methyl ester (carbon tetrachloride); C, maleic anhydride adduct of isomycomycin methyl ester ("nujol mull"); D, perhydro-derivative of maleic anhydride adduct of isomycomycin methyl ester (carbon tetrachloride).

(8) (a) K. W. Hausser, R. Kuhn and G. Seitz, *Z. physik. Chem.*, **B29**, 391 (1935); (b) T. Bruun, C. M. Haug and N. A. Sorensen, *Acta Chem. Scand.*, **4**, 850 (1950); (c) E. R. H. Jones, M. C. Whiting, J. B. Armitage, C. L. Cook and N. Entwistle, *Nature*, **168**, 900 (1951).

(9) An examination of the reported ultraviolet absorption spectra of a number of structurally uncharacterized *Basidiomycetes* antibiotics such as nemotin, nemotinic acid (M. Anchel, *et al.*, *Arch. Biochem.*, **25**, 208 (1950)), agrocybin (F. Kavanagh, *et al.*, *Proc. Natl. Acad. Sci.*, **36**, 102 (1950)) and quadrifidins B2 and B3 (H. M. Doery, *et al.*, *Antibiotics and Chemotherapy*, **1**, 409 (1951)) discloses absorption maxima showing acetylenic spacings indicating conjugated polyacetylenic groupings (8c) in these compounds. It is interesting that nemotin (Anchel, *et al.*) undergoes extensive rearrangement within 1 hour in phosphate buffer *p*H 10 solution at 37° (24 hours in *p*H 7 buffer) evidenced by a pronounced bathochromic shift of the ultraviolet absorption maxima, the acetylenic spacings being retained in the longer wave length region. NOTE ADDED IN PROOF.—After this paper was submitted, Anchel published a note on the correlation of ultraviolet spectra with conjugated acetylenic groupings in compounds derived from fungi. THIS JOURNAL, **74**, 1588 (1952).

(10) (a) K. Bowden, I. Heilbron, E. R. H. Jones and K. Sargent, *J. Chem. Soc.*, 1579 (1947); (b) E. R. H. Jones, *ibid.*, 760 (1950); (c) F. Bohlmann, *Chem. Ber.*, **84**, 785 (1951).

(11) E. R. H. Jones, personal communication.

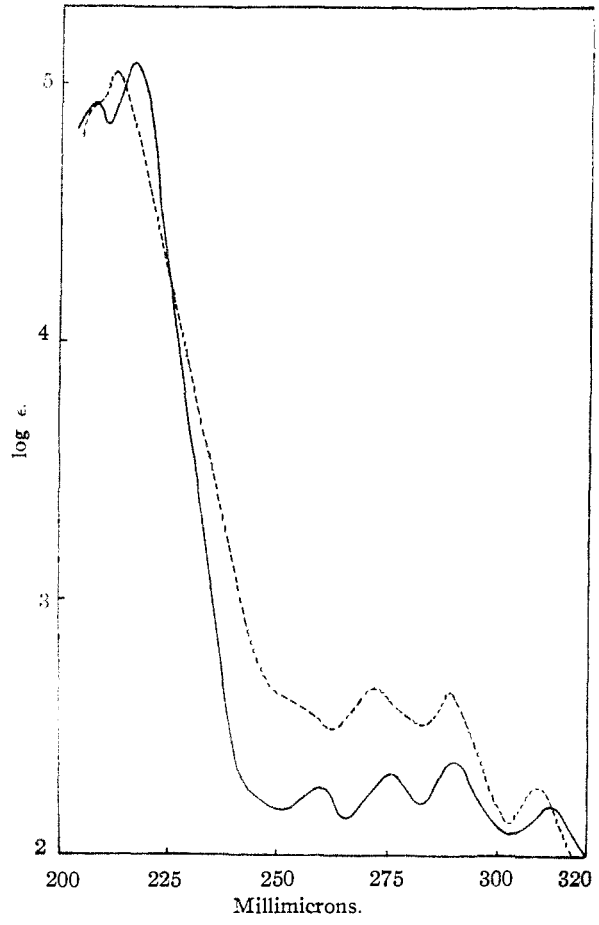


Fig. 2.—Ultraviolet spectra: broken line, maleic anhydride adduct of isomycomycin methyl ester (in methanol); solid line, 1,6-bis-(1-hydroxycyclohexyl)-1,3,5-hexatriyne (in methanol), ref. 10c.

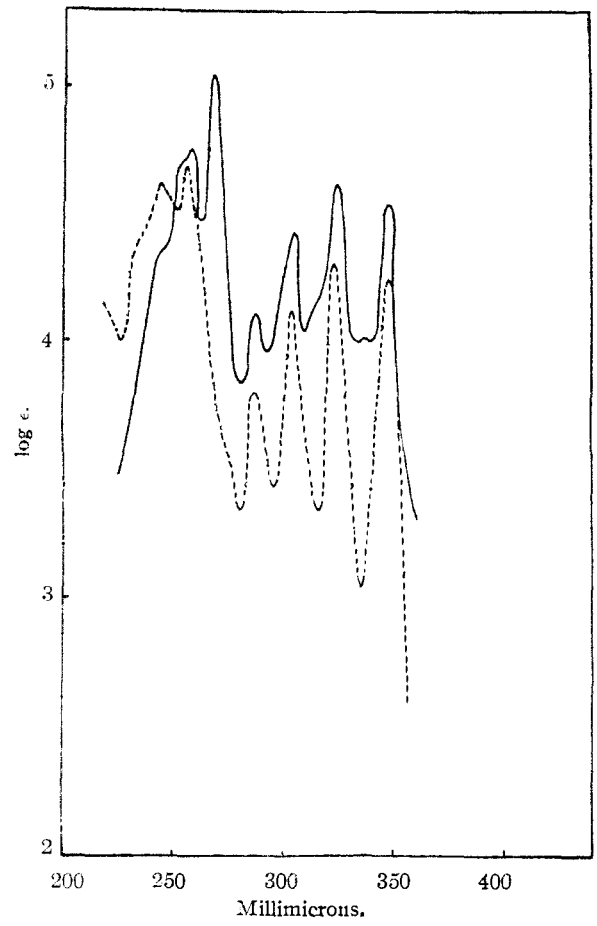


Fig. 3.—Ultraviolet spectra: solid line, isomycomycin methyl ester (in diethyl ether); broken line, dehydromatricaria ester (in hexane), ref. 16.

cm.$^{-1}$.[12] Two moderately strong infrared bands near 1580 and 1620 cm.$^{-1}$ have been previously associated with conjugated C=C stretching absorption.[13] Comparable bands are found in the spectrum of the ester, Fig. 1B. The formation of a maleic anhydride adduct substantiates the presence of a conjugated diene.[14] The possibility of maleic anhydride adding across an en–yne conjugated system[15] is untenable in view of the observed ultraviolet absorption spectrum of the adduct.

The ultraviolet absorption spectra of isomycomycin and its methyl ester closely resemble those of compounds reported to contain dienetriyne chromophores, indicating that the recognized diene and triyne in isomycomycin are conjugated, —C≡C—C≡C—C≡C—CH=CH—CH=CH—. All of the absorption maxima of 2,10-dodecadiene-4,6,8-triyne, CH_3—CH=CH—C≡C—C≡C—C≡C—CH=CH—CH_3, listed by Jones, *et al.*,[8c] compare favorably with those of isomycomycin methyl ester (consult Table I). The general spectrum of dehydromatricaria ester,[16] CH_3—CH=CH—C≡C—C≡C—C≡C—CO_2CH_3 or CH_3—C≡C—C≡C—C≡C—CH=$CHCO_2CH_3$, follows that of isomycomycin methyl ester (consult Table I and Fig. 3). It is of special interest that the carboxyl carbonyl group of dehydromatricaria ester and related compounds[8b] is practically chromophorically equivalent to an ethylenic linkage in regard to the position of the longer wave length group of absorption maxima. In the case of isomycomycin, however, the observed ultraviolet spectrum infers that its carboxyl group is excluded from conjugation.

It is well known[7a,17] that the infrared frequency of the carbonyl stretching mode of saturated acids and esters is located at a measurably higher frequency than the corresponding conjugated compound. Cason, *et al.*,[17c] have demonstrated that β,γ-unsaturated fatty acids and esters likewise exhibit a carbonyl band at a higher frequency than the corresponding α,β-unsaturated compound. The infrared evidence is consistent with the view that the carboxyl group of isomycomycin is unconjugated.

(12) R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 120 (1947).

(13) R. S. Rasmussen and R. R. Brattain, *ibid.*, **15**, 131 (1947).

(14) M. C. Kloetzel, "Organic Reactions," Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1948, pp. 1–59.

(15) (a) E. Dane, O. Hoss, A. W. Bondseil and J. Schmitt, *Ann.*, **532**, 39 (1937); (b) L. W. Butz, A. M. Gaddis, E. W. J. Butz and R. E. Davis, *J. Org. Chem.*, **5**, 379 (1940).

(16) K. Stavholt and N. A. Sorensen, *Acta Chem. Scand.*, **4**, 1567 (1950).

(17) (a) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949); (b) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949; (c) J. Cason, N. K. Freeman and G. Sumrell, *J. Biol. Chem.*, **192**, 415 (1951).

There is no significant difference in the location of the carbonyl absorption band of isomycomycin methyl ester and its saturated derivative (methyl *n*-tridecanoate). Under comparable conditions of spectra determination, esters of known α,β-unsaturation exhibit carbonyl bands at the expected lower frequency than the corresponding saturated compound (consult Table II). In a comparison of dioxane solutions, isomycomycin exhibits a C=O band near 1730 cm.$^{-1}$ which is superimposable on the corresponding band of *n*-tridecanoic acid.

TABLE II

FREQUENCIES OF CARBONYL ABSORPTION MAXIMA[a]

Compound	Wave number (C=O), cm.$^{-1}$
Isomycomycin methyl ester	1733 ± 2
Methyl *n*-tridecanoate	1733 ± 2
Matricaria ester[b]	
$(CH_3—CH=CH—C\equiv C—C\equiv C—CH=CH—CO_2CH_3)$	1724 ± 4
Lachnophyllum ester[b]	
$(CH_3—CH_2—CH_2—C\equiv C—C\equiv C—CH=CH—CO_2CH_3)$	1724 ± 4
Methyl caprate	1733 ± 2
Ethyl 3-methyl-2-nonenoate[c]	1703
Ethyl 3-methyl-3-nonenoate[c]	1727

[a] The infrared spectra were recorded on a Baird infrared spectrophotometer using 5% carbon tetrachloride solutions of the esters in cells of 0.1-mm. thickness. [b] We are indebted to Prof. Nils Andreas Sorensen, Norges Tekniske Hogskole, Trondheim for generous samples of these rare esters. [c] Reproduced from the paper by Cason, *et al.*, ref. 17c. These compounds were determined as 0.2 *M* solutions in chloroform using a 0.1-mm. cell.

In previous reports,[1,4] it was revealed that among its polyunsaturation, mycomycin contains a monosubstituted acetylenic bond and an allenic bond, whereas it has now been established that these linkages are absent in isomycomycin. The pertinent portion of the mycomycin to isomycomycin rearrangement, $HC\equiv C—C\equiv C—CH=C=CH—R \rightarrow CH_3—C\equiv C—C\equiv C—C\equiv C—R$, explains these observed differences.

Isomerization of the asymmetrically substituted allenic bond in mycomycin to an acetylenic bond likewise explains the lack of optical activity of isomycomycin. The known alkali-induced acetylene–allene isomerizations involve equilibrium reactions[18] and substitution undoubtedly affects the reaction conditions and proportion of the principal-product obtained. In the case of alkali-treated mycomycin, it should be pointed out that the compound recognized as isomycomycin crystallizes out of the reaction mixture (as the alkali metal salt in 30–40% yield) and in effect may represent one end-product of a variety of equilibrium reactions.

Confirmation of structure II was obtained by Whitehouse, Eiland and Pepinsky[19] who conducted X-ray structural studies on the crystalline potassium salt of isomycomycin prepared in this Laboratory.

Experimental

Alkali-isomerization of Mycomycin.—The following experiment was conducted at 27° in a nitrogen atmosphere.

To a slurry of crystalline mycomycin (1.0 g.) in carbon dioxide-free distilled water (10 ml.) was added 100 ml. of freshly prepared normal potassium hydroxide. The mycomycin dissolved and the solution gradually became brown in color. Within 10 minutes, a white crystalline precipitate appeared which was recovered after 45 minutes and washed with normal potassium hydroxide followed by ethanol; yield 0.4 g. The salt possessed no definite melting point but slowly decomposed above 150°.

Anal. Calcd for $C_{13}H_9O_2K$: C, 66.07; H, 3.84; equiv. wt., 237. Found: C, 66.19; H, 4.31; equiv. wt. (titration with standard acid), 237.

An aqueous solution of the potassium salt exhibited the following light absorption properties:

λ_{max}^{mu}	248 (infl.)	260	270	289	307	326.5	347.5
$\epsilon \times 10^{-3}$	27	55	90	16	26	41	36

The isomerization of mycomycin was satisfactorily effected utilizing 0.2 to 2.0% concentrations of crystalline mycomycin in 0.5 to 1.5 *N* aqueous potassium, sodium or lithium hydroxide at room temperature. In all cases, the reaction solution became dark brown in color, but the product separated as white crystalline plates. Prolonged standing in alkali was detrimental to both yield and quality of the product. When necessary, the salts were recrystallized from methanol–ethanol. Attempts to recover additional product from the mother liquors invariably gave black tars. The salts were characterized by typical carboxylate infrared absorption[17a] near 1570 cm.$^{-1}$ together with bands near 2200 and 990 cm.$^{-1}$. The potassium salt was favored for preparative purposes because it was usually obtained analytically pure directly from the reaction mixture, presumably because of its slower rate of crystallization.

Isomycomycin Free Acid.—The potassium salt of isomycomycin (1.8 g.) was dissolved in 180 ml. of water; 90 ml. of ether was added and the solution was acidified to *p*H 2 with dilute hydrochloric acid. The ether phase was separated and the aqueous raffinate was extracted twice more with 40-ml. portions of ether. The ether extracts were combined, dried over anhydrous sodium sulfate, filtered and the solvent was removed by distillation at reduced pressure. The dry residue (1.45 g.) was crystallized from ether–hexane as white needles (1.1 g.). The crystals possessed no definite melting point but slowly decomposed above 140°.

Anal. Calcd. for $C_{13}H_{10}O_2$: C, 78.76; H, 5.08; one C-methyl, 7.6; neut. equiv., 198. Found: C, 78.87; H, 5.43; C-methyl (Kuhn–Roth), 6.3, 6.8; neut. equiv., 198.

A diethyl ether solution of the free acid exhibited the following light absorption properties:

λ_{max}^{mu}	246 (infl.)	257.5	267	287.5	305.5	324	347
$\epsilon \times 10^{-3}$	24	58	110	14	27	41	34

Though isomycomycin is relatively more stable than mycomycin, the crystals discolor after several hours at room temperature (27°). For prolonged storage all samples were sealed in ampules under nitrogen and kept at Dry Ice temperature. Whereas mycomycin polymerizes rapidly in liquid ammonia,[4] isomycomycin can be recovered as the ammonium salt.

Hydrogenation of Isomycomycin. Identification of *n*-Tridecanoic Acid.—Isomycomycin (200 mg., 1.01 millimoles) was hydrogenated in 50 ml. of ethyl alcohol at atmospheric pressure and 27° over 200 mg. of previously reduced platinum oxide catalyst. The hydrogen uptake was rapid and ceased after 30 minutes with a consumption of 178 ml. of hydrogen (S.T.P.) or 7.95 millimoles. The platinum was filtered and the solvent was removed by distillation at reduced pressure. The residue (216 mg.) crystallized directly, freezing point 39–40°. The infrared spectrum of the reduction product was identical with that of *n*-tridecanoic acid. Recrystallization from acetonitrile raised the freezing point to 40.3°; mixed freezing point with *n*-tridecanoic acid was undepressed.

Anal. Calcd. for $C_{13}H_{26}O_2$: C, 72.80; H, 12.13; neut. equiv., 214. Found: C, 72.90; H, 12.02; neut. equiv., 214.

The *p*-phenylphenacyl ester prepared according to Price and Griffith[20] melted at 86.6–87.0°, undepressed by

(18) (a) T. L. Jacobs, "Organic Reactions," Vol. V, John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 13–17; (b) T. L. Jacobs, R. Akawie and R. G. Cooper, THIS JOURNAL, **73**, 1273 (1951).

(19) T. Whitehouse, P. F. Eiland and R. Pepinsky, *Acta Cryst.*, in press.

(20) D. Price and R. Griffith, THIS JOURNAL, **62**, 2884 (1940).

admixture with an authentic sample of *p*-phenylphenacyl *n*-tridecanoate, m.p. 86.6–87°.

Isomycomycin Methyl Ester.—Isomycomycin (900 mg.) dissolved in 100 ml. of ether was treated with an excess of ethereal diazomethane. The solution was evaporated to dryness and the residual solid was recrystallized from hexane to yield 800 mg. of the methyl ester of isomycomycin, m.p. 69–70°.

Anal. Calcd. for $C_{14}H_{12}O_2$: C, 79.24; H, 5.66; CH_3O, 14.62. Found: C, 79.23; H, 5.71; CH_3O, 14.01.

See Table I and Figs. 1 and 3 for light absorption data.

Hydrogenation of isomycomycin methyl ester (110 mg., 0.52 millimole) in 10 ml. of ethyl acetate over 200 mg. of previously reduced platinum oxide catalyst at atmospheric pressure required 93 ml. of hydrogen (S.T.P.) equivalent to 4.11 millimoles or 7.9 moles per mole of ester. The infrared spectrum of the reduction product was indistinguishable from that of methyl *n*-tridecanoate.

Maleic Anhydride Adduct of Isomycomycin Methyl Ester.—Milliequivalent portions of isomycomycin methyl ester (212 mg.) and maleic anhydride (98 mg.) were mixed and heated at 69–72° for 40 minutes in a sealed tube. Unreacted materials were extracted with ether leaving a white, crystalline residue (125 mg.), m.p. 165–170° (dec.). Recrystallization from acetone–hexane yielded crystalline plates, m.p. 177–178° (dec.). See Table I and Figs. 1 and 2 for light absorption properties.

Anal. Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55; CH_3O, 10.00. Found: C, 69.59; H, 4.64; CH_3O, 10.89.

The same product was isolated in comparable yield using a fourfold excess of maleic anhydride.

Hydrogenation of Maleic Anhydride Adduct of Isomycomycin Methyl Ester.—The maleic anhydride adduct of isomycomycin methyl ester (145 mg., 0.47 millimole) dissolved in 15 ml. of ethyl acetate was hydrogenated at atmospheric pressure and 27° over 100 mg. of previously reduced Adams platinum oxide catalyst. The compound consumed 76 ml. of hydrogen (S.T.P.) equivalent to 3.4 millimoles or 7.2 moles per mole of adduct. The catalyst was removed by filtration and the filtrate evaporated *in vacuo*. A colorless oil remained which was evaporatively distilled at approximately 1 mm. pressure and 160° block temperature. The distillate, $n^{25}D$ 1.4780, was too viscous for a precise density determination.

Anal. Calcd. for $C_{15}H_{28}O_5$: C, 66.64; H, 8.70. Found: C, 66.86; H, 8.73.

Acknowledgments.—The authors wish to express deep appreciation to Dr. Wilbur A. Lazier for his active interest in the investigation. We are indebted to Dr. John Means and Mr. Glenn B. Hess for the analytical and spectral data.

BROOKLYN 6, N. Y.

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

The Structure of Veratramine

BY CH. TAMM[1] AND O. WINTERSTEINER

RECEIVED JANUARY 31, 1952

Chemical proof for the existence in veratramine of a preformed benzeneoid ring has been adduced by the conversion of triacetyldihydroveratramine to an aromatic nitro derivative. N-Acetylveratramine on Oppenauer oxidation yielded an α,β-unsaturated monoketone which is undoubtedly a Δ^4-3-ketone, thus confirming the conclusion reached by Jacobs and Sato[5] regarding the 5,6-position of the double bond from the corresponding experiment on the free base. Chromic acid oxidation of triacetyldihydroveratramine afforded a compound to which the indanone structure VI is assigned in consideration of the identity of its ultraviolet absorption spectrum with that of the jervine derivative V. Accordingly the perhydrobenzfluorene structure III is proposed for veratramine. Several other new derivatives of the alkaloid are described and formulated on this basis.

Our knowledge of the chemistry of the secondary base veratramine, $C_{27}H_{39}O_2N$, which was first isolated by Saito[2] from *Veratrum grandifolium* Loes. fil., is largely based on the recent studies of Jacobs and his collaborators[3–5] who secured it from the domestic species *Veratrum viride* Aiton. Both oxygen atoms are present as acylable hydroxyl groups.[3] Veratramine contains a benzenoid ring, as evidenced by its ultraviolet absorption spectrum,[3] which resembles that of neoergosterol, and furthermore an ethylenic bond demonstrable by catalytic hydrogenation.[2,3] On selenium dehydrogenation[4] the alkaloid yielded a basic fragment C_6H_7ON later identified by synthesis as 3-methyl-5-hydroxypyridine,[5] and in the neutral fraction a hydrocarbon $C_{22}H_{20}$ indistinguishable from one obtained in the same reaction from the closely related secondary base jervine, the main alkaloid of *Veratrum viride*,[6] and believed to be either a homolog of 1,2-benzfluorene[6] or of chrysene.[4]

In 1949 Jacobs and Sato[4] proposed, on the basis of the facts then available, structure I for veratramine. This formula became untenable when it was found more recently[5] that veratramine could be converted by Oppenauer oxidation to an amorphous ketonic base (λ_{max}^{alc} 230 mμ, log ϵ 4.3) which was crystallizable as the hydrochloride and yielded an amorphous monoxime. This compound is undoubtedly Δ^4-veratramin-3-one, as the allylic alcohol obtained from it by Meerwein–Ponndorf reduction gave a positive Rosenheim test. It is thus clear that the double bond occupies the 5,6-position (4,5 being excluded by other evidence), and formula I was accordingly abandoned in favor of the perhydrochrysene structure, II, in which ring D is aromatic. The latter postulate derives its support from the fact already mentioned that veratramine on selenium dehydrogenation yielded its nitrogenous ring as 3-methyl-5-hydroxypyridine, whereas all other veratrum alkaloids gave rise in this reaction to pyridine derivatives carrying in position 6 an ethyl group representing carbon atoms 20 and 21 of the skeleton (*i.e.*, 3-methyl-6-ethylpyridine, and in the case of jervine, 3-methyl-5-hydroxy-6-ethylpyridine). It is reasoned that attachment, in veratramine, of the side chain to the aromatic nucleus would result in scission of the

(1) Research Fellow 1949–1950, Schweizerische Stitfung für Stipendien auf dem Gebiete der Chemie.

(2) K. Saito, *Bull. Chem. Soc. Japan*, **15**, 22 (1940).

(3) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945).

(4) W. A. Jacobs and Y. Sato, *ibid.*, **181**, 55 (1949).

(5) W. A. Jacobs and Y. Sato, *ibid.*, **191**, 71 (1951).

(6) W. A. Jacobs, L. C. Craig and G. I. Lavin, *ibid.*, **141**, 51 (1941).