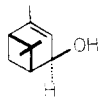
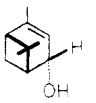
cyclobutane ring having virtually no effect beyond this, and so should make rotatory contributions of ±130°. The calculated rotations are reasonably close to those observed considering the assumptions used in the calculations. The predicted difference in rotation of the epimers (Δ[M] −260°) should be compared with that observed (−226°). Rotations can be calculated for the verbenols (XXIX, XXX)[27] in a similar way. The value calculated for the *trans* isomer XXX is in good agreement with that found for the isomer to which a *trans* configuration has been assigned. The observed value for the presumed *cis*-verbenol is badly out of line with that calculated; it is, however, quite close to that expected for a 1:1 mixture of the two isomers (+80°, observed +100°). This case is to be compared with that of the carvotanacetols (above).



cis

+80 − 160 = −80°
"Obsd. [M]D" +100°[27]
(see text)

XXX



trans

+80 + 160 = +240°
Obsd. [M]D +257°[27]

XXXI

It might, at first thought, be expected that one could calculate the rotatory effects resulting from the introduction of a double bond into the steroid nucleus. An inspection of models suggests, however, that a fused cyclohexene ring may prefer a conformation in which five of the ring atoms are planar and in which three atoms have true axial and equatorial bonds (XXXII). It is to be noted that this form is only slightly altered, relative to the semi-chair form; it would be expected to have the same sign of rotation as the corresponding semi-chair form, but a different magnitude of rotation. Conversely, if the cyclohexene ring has the semi-chair conformation it may distort the ring fused to it and produce an additional rotatory shift. Accordingly, predictions in this series can be expected to be only rough and approximate.[28] As seen in Table V, the present simple treatment gives correct predictions of the *sign* of rotatory change in every case, but the predictions of magnitude are, more often than not, rather poor. In the case of the Δ⁴-steroids, ring A can assume either of the two ring conformations IIIa or IIIb; the observed value is close to that calculated on the assumption that the two forms are present in equal amounts. The results suggest that caution be used in this application of the present method to fused ring compounds.

TABLE V

CHANGES IN MOLECULAR ROTATION ON INTRODUCTION OF A DOUBLE BOND INTO THE STEROID NUCLEUS

Position of C=C		Ring	Rotatory contributions α-Substituent	β-Substituent *Endo*	β-Substituent *Exo*	Δ(C=C) → (CH–CH) Calcd.	Obsd.
A/B *trans*							
1:2		−160	0	0	+80	− 80	− 47[c]
2:3		+160	0	−80	0	+ 80	+167[c]
3:4		−160	+130	+80	0	+ 50	+124[c]
6:7		+160	−260	−80	−80	−260	−402[c]
8:9		0	+130	+80	0	+210	+ 96[d]
A/B *cis*							
1:2		+160	0	0	+80	+240	
2:3		−160	0	+80	0	− 80	− 24[d]
3:4		+160	−130	−80	0	− 50	− 44[d]
6:7		+160	0	−80	−80	0	
8:9		0	+130	0	0	+130	
Both series							
4:5	a	+160	+130	0	0	+130	+153[c]
	b	−160	+130	0	0		
5:6		−160	−130	0	0	−290	−294[c]
11:12[a]		−160	+130	0	+80	+ 50	+33[d]
15:16[a]		0	0	0	0	0	
16:17[b]		0	0	0	0	0	+ 31[d]

[a] Substituent at C_{17}. [b] No substituent at C_{17}. [c] Data for cholestenes; Δ-values calculated from [M]D for cholestane. +91; data of R. B. Turner, W. R. Meador and R. E. Winkler, THIS JOURNAL, **79**, 4122 (1957). [d] Average Δ-values as tabulated by W. Klyne in E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955, p. 111.

XXXII

(27) One isomer, having a high rotation, is obtained by autoxidation of (+)-α-pinene and isolated *via* the *p*-nitrobenzoate. Another material, presumed to be isomeric, was obtained *in an impure state* from the *p*-nitrobenzoate of the product of Ponndorf reduction of verbenone. Both products could be oxidized to the same verbenone, *but there is no evidence that the last named alcohol was not a mixture of epimers*. Relative configurations were assigned by use of the Auwers–Skita rules and by a comparison of rates of esterification [H. Schmidt, L. Schulz and W. Doll, *Chem. Zentr.*, **111**, II, 3038 (1940); L. Schulz and W. Doll, *ibid.*, **115**, II, 755 (1944).]

(28) The empirical constants used to this point do not allow prediction of the rotation shift for introduction of a double bond at 7:8, 9:11 or 14:15. Uncertainty as to the conformation of ring D prevents a prediction for introduction of a double bond at 8:14.

LAFAYETTE, IND.

[CONTRIBUTION FROM THE RESEARCH DIVISION, PARKE, DAVIS & COMPANY]

Chemistry of Streptimidone, A New Antibiotic

BY ROGER P. FROHARDT, HENRY W. DION, ZBIGNIEW L. JAKUBOWSKI, ALBERT RYDER, JAMES C. FRENCH AND QUENTIN R. BARTZ

RECEIVED APRIL 17, 1959

A new antibiotic, streptimidone ($C_{16}H_{23}NO_4$), has been obtained from the culture filtrates of a *Streptomyces*. The characteristic physical properties of the antibiotic have been determined. Degradative studies have demonstrated that streptimidone is 3-(2-hydroxy-7-methyl-5-methylene-4-oxo-6-nonenyl)-glutarimide. A novel cross-conjugated chromophore is proposed for the dienone.

A new antibiotic, streptimidone, has been obtained from the culture filtrates of a *Streptomyces*. It resembles actidione,[1] inactone,[2] fermicidin,[3] E73[4] and streptovitacins A and B[5] in some respects but can be easily discerned from these substances by its chemical and biological properties.

The compound was obtained in the following manner: (1) extraction of the culture filtrate at *p*H 5 with ethyl acetate; (2) washing of the concentrated extract with dilute aqueous sodium carbonate, dilute acid and water; (3) precipitation of the crude streptimidone from ethyl acetate solution with petroleum ether; (4) chromatography of the precipitated crude antibiotic on activated carbon from acetone solution; (5) crystallization from an acetone–isopropyl ether solution.

Streptimidone crystallizes from an acetone–isopropyl ether solution as fine colorless needles melting at 72–73°. It is soluble in methanol, ethanol, acetone, ethyl acetate, chloroform, only slightly soluble in water and ethyl ether, and insoluble in petroleum ether. A 25-plate Craig countercurrent extraction, using 1-butanol, cyclohexane, water (1:4:5, K = 1.10), demonstrated that three times crystallized streptimidone consisted of only one component. The antibiotic in aqueous solution is most stable at about *p*H 4.5. After 24 hr. at 25°, its activity against *Kloeckera africana* M1570 is decreased by approximately 40% at *p*H 8, in contrast to a decrease of about 10% at *p*H 1. After two weeks of sunlight at room temperature, the crystalline compound becomes yellow and oily. It polymerizes to an oily gum when lyophilized from aqueous solution.

Streptimidone possesses the empirical formula $C_{16}H_{23}NO_4$ (293.35). Molecular weight determination by titration in water solution gives a value of 290 (pK'a 11.2), by the isothermal distillation technique, a value of 307.

The specific rotation is $[\alpha]^{28}D$ +238° (c 0.5% in water), $[\alpha]^{27}D$ +245° (c 0.5% in chloroform). The compound exhibits a characteristic ultraviolet spectrum (Fig. 1) in methanol with maxima at 232 mμ (ε 23,100) and 291 mμ (ε 790); in water, with maxima at 231 mμ (ε 19,600) and 289 mμ (ε 1,030). The infrared spectrum (Fig. 2, KBr wafer) shows strong absorption at 2.82, 2.90, 3.15, 3.24, 3.41, 5.85, 5.88, 5.96, 6.09, 6.21, 7.28, 7.75, 8.09, 8.50, 8.65, 9.12, 9.46, 9.74, 9.85, 10.11, 11.12, 11.25 and 11.45 μ.

Distillation of the antibiotic with alkali liberates all of the nitrogen as ammonia. A Kuhn–Roth C-methyl determination gives a value of 1.45. The antibiotic forms a monoacetate and contains one carbonyl group according to the hydroxylamine assay of Siggia[6]; the crystalline oxime, dissolved in methanol, exhibits a single maximum in the ultraviolet at 233 mμ (ε 24,600).

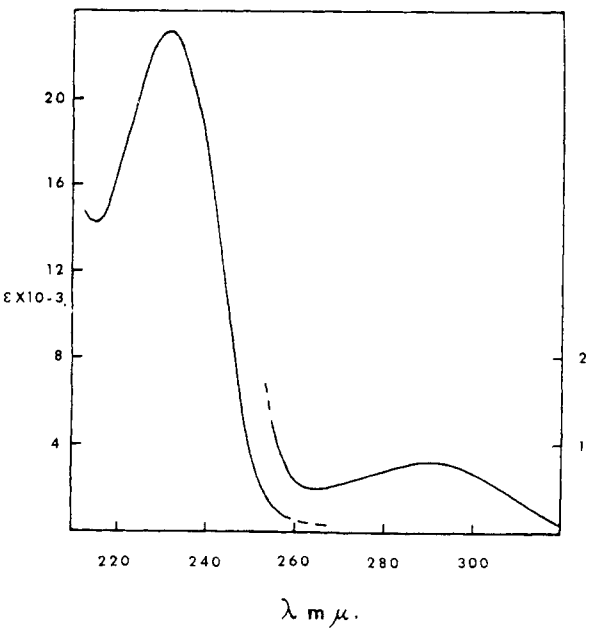


Fig. 1.—Ultraviolet absorption spectrum of streptimidone in methanol.

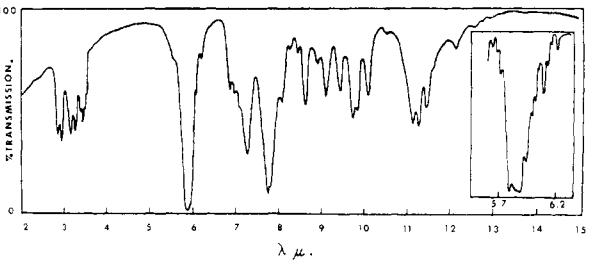


Fig. 2.—Infrared spectrum of streptimidone in compressed potassium bromide; expanded portion run in pyridine solution.

The antibiotic quickly decolorizes bromine as well as an aqueous permanganate solution; it gives a positive reaction in the following tests: (1) *m*-phenylenediamine for α,β-unsaturated aldehydes and ketones[7]; (2) ferric hydroxamate directly; (3) sodium nitroprusside for methyl ketones.[8] It gives a negative reaction in the following tests: ninhydrin, periodate, Tollens and titanium chloride for enediols and enols of 1,3-diketones.[9]

The compound absorbs one, two or three moles of hydrogen depending on the catalyst or solvent used. In ethanol solution with palladium on calcium carbonate, it takes up one mole of hydrogen to give dihydrostreptimidone, $C_{16}H_{25}NO_4$, which melts at 47–49° and exhibits a single maximum in the ultraviolet in methanol solution at 289 mμ

(1) B. E. Leach, J. H. Ford and A. J. Whiffen, THIS JOURNAL, **69**, 474 (1947); E. C. Kornfeld, R. G. Jones and T. V. Parke, *ibid.*, **71**, 150 (1949).

(2) J. Preud'homme and M. Dubost, Paper "Intern. Cong. of Organic Chemistry," Zurich, 1955; R. Paul and S. Tchelitcheff, *Bull. soc. chim. France*, 1316 (1955).

(3) S. Igarasi and S. Wada, *J. Antibiotics (Japan)*, **B7**, 221 (1954).

(4) K. V. Rao and W. P. Cullen, Abstracts of Papers, 134th Meeting, American Chemical Society, Chicago, Illinois, Sept. 1958, pp. 22-23-O; K. V. Rao, *ibid.*, p. 23-O.

(5) T. E. Eble, M. E. Bergy, C. M. Large, R. R. Herr and W. G. Jackson in H. Welch and F. Marti-Ibañez, "Antibiotics Annual," Medical Encyclopedia, Inc., New York, N. Y., 1958–1959, pp. 555–559; R. R. Herr pp. 560–564.

(6) S. Siggia, "Quantitative Organic Analysis *via* Functional Groups," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 16–19.

(7) R. B. Wearn, W. M. Murray, Jr., M. P. Ramsey and N. Chandler, *Anal. Chem.*, **20**, 922 (1948).

(8) F. Feigl, "Spot Tests: v. II Organic Applications," 4th Ed., Elsevier Publishing Co., New York, N. Y., 1954, p. 160.

(9) F. Weygand and E. Csendes, *Chem. Ber.*, **85**, 45 (1952).

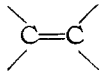
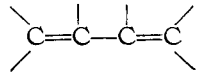
with an ϵ value of 160, indicative of a β,γ-unsaturated ketone.[10] The infrared spectrum of the reduced compound shows the absence of maxima at 6.21, 10.11 and 11.12 μ which are present in streptimidone. The compound still rapidly decolorizes bromine and aqueous permanganate solutions. A Kuhn–Roth C-methyl determination gives a value of 2.16, an increase of 0.71 over the parent compound, which fact coupled with the infrared spectrum indicates the reduction of a methylene group. In 0.1 *N* sodium hydroxide, dihydrostreptimidone shows a shift in the ultraviolet spectrum from a maximum at 285 mμ (ϵ 280) to 231 mμ (ϵ 4,500) after standing at room temperature for 24 hr., indicating a shift of a double bond into conjugation with a carbonyl group.

With palladium-on-carbon in ethanol solution, streptimidone takes up two moles of hydrogen to give tetrahydrostreptimidone ($C_{16}H_{27}NO_4$), m.p. 56.5–57°, which exhibits a maximum in the ultraviolet spectrum at 286 mμ (ϵ 42), typical of simple carbonyl absorption. The infrared spectrum has major maxima at 2.80, 2.91, 3.34, 5.84, 6.84, 7.23, 7.80, 7.96, 8.68 and 9.58 μ. Tetrahydrostreptimidone possesses a weakly acidic group with a pK'a of 11.2 in water. It forms a monoöxime and a monoacetate and does not quickly decolorize bromine or aqueous permanganate solutions. Thus, two double bonds have been reduced in the catalytic reduction of streptimidone with 5% palladium on carbon in ethanol solution.

With platinum oxide in acetic acid solution, streptimidone is hydrogenated to hexahydrostreptimidone ($C_{16}H_{29}NO_4$), m.p. 95–102°, which does not exhibit carbonyl absorption in the ultraviolet spectrum nor does it form an oxime. The compound does not decolorize bromine or aqueous permanganate solutions, nor does it reduce periodate. The infrared spectrum of hexahydrostreptimidone exhibits maxima at 2.84, 2.90, 3.35, 5.83, 6.83, 6.97, 7.23, 7.36, 7.95, 8.67 and 9.15 μ.

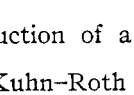
Streptimidone, dissolved in aqueous dioxane, is reduced with sodium borohydride to give an oil exhibiting a single maximum in the ultraviolet at 230 mμ (ϵ 23,600), a spectrum typical of dienes. The reduction product quickly decolorizes bromine and aqueous permanganate solutions, does not form a semicarbazone, but gives a diacetate. Thus, treatment of the antibiotic with sodium borohydride effected the reduction of a carbonyl group to an alcohol.

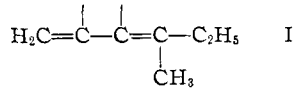
Streptimidone in methanol solution takes up two moles of ozone at −80°. Hydrolysis of the ozonide with aqueous ferrous sulfate yields 0.56 mole of methyl ethyl ketone, 0.16 mole of formaldehyde and 0.11 mole of acetaldehyde; the carbonyl compounds were isolated as their 2,4-dinitrophenylhydrazones.

The foregoing reduction and ozonolysis data demonstrate that streptimidone contains two  groups. That these two double bonds are conjugated to give a diene unit 

(10) R. C. Cookson and N. S. Wariyar, *J. Chem. Soc.*, 2302 (1956).

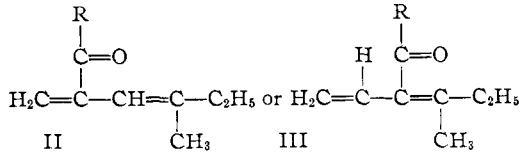
is shown by: (a) the ultraviolet spectrum of the antibiotic with a maximum at 232 mμ and ϵ value of 23,100; (b) the bands in the infrared spectrum of streptimidone at 6.09 and 6.21 μ; (c) the ultraviolet spectrum of the borohydride reduction product with a maximum at 230 mμ and ϵ value of 23,600.

Catalytic reduction of the antibiotic with palladium on calcium carbonate brings about the reduction of a  group as indicated by a Kuhn–Roth C-methyl determination and the disappearance of the 11.12 μ band in the infrared spectrum. Thus, one end of the diene chromophore is a methylene group. The formaldehyde, obtained in low yield in the ozonolysis of streptimidone, also confirms the presence of the end methylene group. The other end of the diene chain is a *sec*-butylene group since the main product from the ozonolysis is methyl ethyl ketone. The basic diene unit is thus I



I

Streptimidone is shown to contain a carbonyl group by means of: (1) a quantitative carbonyl determination by means of the Siggia oxime method[6]; (2) the ultraviolet spectrum of tetrahydrostreptimidone; and (3) the small band in the expanded infrared spectrum of streptimidone at 5.96 μ. A positive *m*-phenylenediamine test for α,β-unsaturated ketones and the two complementary brands in the infrared spectrum at 5.96 and 6.09 μ indicate that the carbonyl group is α,β-unsaturated. Thus, the basic chromophore of streptimidone could be either II or III.



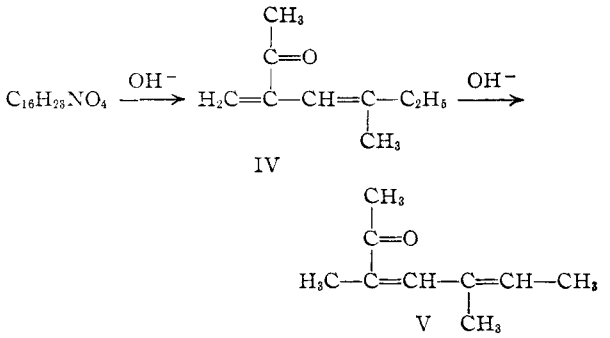
II III

The placing of the carbonyl branch follows since, as mentioned previously, the addition of one mole of hydrogen to streptimidone (palladium on calcium carbonate) effects the reduction of a methylene group and the product is a β,γ-unsaturated ketone with a maximum at 289 mμ and ϵ value of 160. Thus, II is the structure compatible with the experimental data.

Alkaline hydrolysis of streptimidone gives the following products: (1) one equivalent of ammonia identified as the *p*-hydroxy-azobenzene-sulfonate salt; (2) a steam volatile oil, $C_9H_{14}O$, whose constitution depends on the severity of the alkaline treatment; and (3) an acid fraction which, when chromatographed on paper using 0.75 *N* 80% aqueous ethanolic ammonium hydroxide as solvent, gives the identical acid components obtained from the alkaline hydrolysis of actidione.

Treatment of streptimidone with 1 *N* sodium hydroxide at room temperature for 2 hr. gives a volatile oil fraction which exhibits maxima in the ultraviolet in ethanol at 232 mμ (ϵ 16,900) and 279 mμ (ϵ 4,400). The oil gives a negative Tollens,

a positive iodoform and reacts with 2,4-dinitrophenylhydrazine.[11] When this information is applied to structure II, it follows that the main portion of the oil is 5-methyl-3-methylene-4-hepten-2-one (IV). Further treatment of IV with 6 *N* sodium hydroxide at 100° gives the linearly conjugated 3,5-dimethyl-3,5-heptadien-2-one (V).



IV

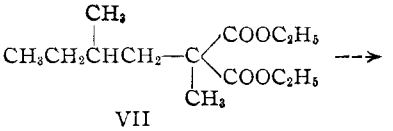
V

Because of this possible shifting of the double bonds, effected by alkali, the homogeneity of the above volatile oil obtained in the 1 *N* sodium hydroxide hydrolysis is doubtful.

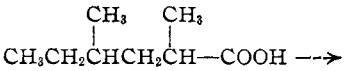
When dihydrostreptimidone was dissolved in 1 *N* sodium hydroxide and the solution immediately steam distilled, the cleavage products obtained are the same as obtained with streptimidone except for the volatile oil ($C_9H_{16}O$). The latter possesses a maximum in the ultraviolet at 281 mμ (ε 320) and gives a 2,4-dinitrophenylhydrazone which melts at 94–96° and exhibits a maximum in the ultraviolet at 360 mμ (ε 21,900). The above ultraviolet data on the volatile oil demonstrates that the carbonyl compound has a double bond in the β,γ-position.[10] The oil is thus 3,5-dimethyl-4-hepten-2-one. Treatment of the latter with 1 *N* methanolic sodium hydroxide for 24 hr. at room temperature shifts the maximum from 281 to 230 mμ with an increase in absorptivity, denoting a shifting of the double bond β,γ to the carbonyl group to an α,β-position. Ozonolysis of the above original oil in methanol solution at −80° gives methyl ethyl ketone. This is further proof of the cross-conjugated structure II proposed earlier.

Alkaline hydrolysis of tetrahydrostreptimidone with 3 *N* sodium hydroxide at 100° gives the same hydrolytic products as obtained with streptimidone except for the volatile oil. The latter oil $C_9H_{18}O$, b.p. 174–175°, exhibits a maximum in the ultraviolet at 282 mμ (ε 34), possesses a strong carbonyl band in the infrared at 5.83 μ, forms a monoxime, a semicarbazone and a 2,4-dinitrophenyl hydrazone (m.p. 72–73°) with a maximum in ethanol in the ultraviolet at 360 mμ (ε 22,400). A positive iodoform and a negative Tollens test are further evidence that the volatile oil is 3,5-dimethyl-2-heptanone (VI).

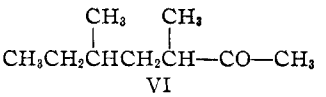
The structure of the latter was proven by an independent synthesis. The starting material was primary "active" amyl alcohol of approximately 97% optical purity. The corresponding bromide was condensed with diethylmethylmalonate to yield the diester VII. Saponification followed by decarboxylation gave 2,4-dimethylhexanoic acid (VIII). The latter acid was then con-



VII



VIII



VI

verted, *via* its acid chloride by means of the cadmium Grignard reaction, to the desired ketone VI. The synthetic product compared very closely with the boiling point and refractive index of the C_9-ketone isolated from the reaction of tetrahydrostreptimidone with sodium hydroxide, and the infrared spectra of these two compounds were essentially superimposable. However, whereas the semicarbazone and the 2,4-dinitrophenylhydrazone of the ketone from tetrahydrostreptimidone were sharp melting compounds, the same derivatives of the synthetic ketone melted over a definite range since a mixture of diastereoisomers was inevitable. Nevertheless, satisfactory analyses for the synthetic semicarbazone and 2,4-dinitrophenylhydrazone were obtained and the infrared spectra of these derivatives were identical with the corresponding compounds derived from tetrahydrostreptimidone.

Treatment of hexahydrostreptimidone with 6 *N* sodium hydroxide for 3 hr. at 100° gives ammonia, no volatile oil and an amorphous acid component exhibiting no maxima in the ultraviolet.

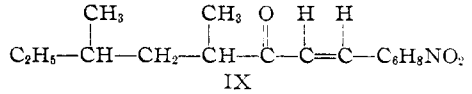
The ease with which streptimidone and its dihydro- and tetrahydro-derivatives are hydrolyzed with alkali, and the resistance of hexahydrostreptimidone to alkaline cleavage is indicative of a β-hydroxy ketone group. Further proof for the presence of a β-hydroxy ketone in streptimidone is obtained by refluxing tetrahydrostreptimidone with phosphorus pentoxide in benzene solution for 15 minutes; the resulting anhydro product in methanol exhibits maxima in the ultraviolet at 225 mμ (ε 12,400) and 322 mμ (ε 56). The infrared spectrum with bands at 6.00 and 6.12 μ is indicative of an

(11) Treatment of the above oil with 2,4-dinitrophenylhydrazine gives two different compounds. Component A, the minor product, is a red hydrazone, analyzes for $C_{15}H_{18}N_4O_4$, melts at 163–164°, and exhibits a maximum in the ultraviolet in ethanol at 382 mμ (ε 28,300). Component B, the major product, is yellow, analyzes for $C_{15}H_{18}N_4O_4$, melts at 113–114° and exhibits a maximum in the ultraviolet in ethanol at 360 mμ (ε 22,400). The latter compound is probably a pyrazoline rearrangement product of IV [R. C. Elderfield, "Heterocyclic Compounds," Vol. V, John Wiley and Sons, Inc., New York, N. Y., 1957, pp. 57–64].

Component A may be the hydrazone of V although the ultraviolet spectrum with a maximum at 382 mμ is not compatible with a doubly unsaturated ketone like V. It is possible that component A may be the 2,4-dinitrophenylhydrazone of cyclized V, *i.e.*, 2,4,5-trimethyl-2-cyclohexen-1-one. Since the precise formulation of these derivatives is not crucial to the structure determination of streptimidone, the exact nature of these compounds was not investigated further at this point. In any case, the parent carbonyl compound for both components is $C_9H_{14}O$.

Cleavage of the antibiotic by heating with 6 *N* sodium hydroxide for 1 hr. at 100° gives a volatile oil fraction (V) exhibiting maxima in the ultraviolet at 226 mμ (ε 4,800) and 279 mμ (ε 13,400). Only one 2,4-dinitrophenylhydrazone was obtained from the latter oil and that corresponded to the red hydrazone component A described above.

α,β-unsaturated ketone. Phosphorus pentoxide thus dehydrates the β-hydroxy ketone to yield an α,β-unsaturated ketone, which must have, according to Woodward's rule,[12] structure IX. The double bond can only be mono-substituted. The



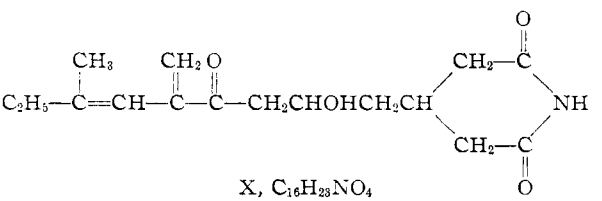
IX

substitution must be placed on the β-carbon atom since a methyl ketone is formed upon alkaline cleavage of tetrahydrostreptimidone.

Since tetrahydrostreptimidone, containing a β-hydroxy ketone, is easily hydrolyzed with base, it was refluxed with benzylamine to give a crystalline product ($C_{21}H_{25}N_3O_2$) which is identical with that obtained by Kornfeld, *et al.*,[1] by refluxing actidione with benzylamine. The identity of the two degradation products was proved by means of a mixed melting point determination, comparison of the ultraviolet and infrared spectra, X-ray powder diagrams and elemental analyses. This demonstrates the presence of the β-ethylglutarimide moiety in streptimidone.

The infrared spectrum of streptimidone, especially the bands at 2.90, 5.85 and 5.88 μ, the pK'a of 11.2 in water, plus the benzylamine product from tetrahydrostreptimidone indicate that the ammonia evolved upon alkaline hyrolysis of the antibiotic is present in the intact molecule as part of a cyclic imide.

Thus, on the basis of the evidence presented, streptimidone (X) is 3-(2-hydroxy-7-methyl-5-methylene-4-oxo-6-nonenyl)-glutarimide.



X, $C_{16}H_{23}NO_4$

Experimental

Assay.—The inhibitory activity of streptimidone against *Kloeckera africana* M1570 (NRRL Y-1274) was employed for assay purposes during the preparative studies. These determinations were carried out using a paper-disk tray procedure.[13] A unit of *Kloeckera africana* activity is defined as that amount of streptimidone which, when applied under standard conditions, produces a zone of inhibition of 16.1 mm. Crystalline streptimidone assays 14 units per mg.

Preparation of Streptimidone.—The fermentation broth (250 liters), adjusted to *p*H 5 with sulfuric acid, was filtered through a pad of Celite 545. The filtrate was extracted twice with 40-liter portions of ethyl acetate. The extract was concentrated *in vacuo* to 3 liters and washed successively three times with 300-ml. portions of each of the following: 1 *M* aqueous sodium carbonate, 0.1 *M* hydrochloric acid and water. The ethyl acetate solution was concentrated to 0.4 liter and the crude streptimidone precipitated from solution with 3.6 liters of petroleum ether. The crude product was dissolved in 370 ml. of acetone and chromatographed on a column of 1200 g. of Darco G-60 and 1200 g. of Celite 545 prepared in acetone. Acetone was used as developer and eluant. The fractions possessing maximum *K. africana* activity were combined and concentrated to 835 ml.; 5 volumes of isopropyl ether were added and the clear solution was chilled. The resulting crystals were filtered and dried. For analytical purposes, the streptimidone was recrystallized several times from acetone–isopropyl ether solution; yield 56.7 g.; m.p. 72–73°; λ_{max}^{MeOH} 232 mμ (*a* 78.8), 291 mμ (*a* 2.7).

Anal. Calcd. for $C_{16}H_{23}NO_4$: C, 65.50; H, 7.90; N, 4.78; O, 21.82; mol. wt., 293.35. Found: C, 65.66; H, 8.10; N, 4.78; O, 22.28; mol. wt., 290 (titration), 307 (isothermal distillation).

Streptimidone Acetate.—Streptimidone (968 mg.) was dissolved in 5 ml. of acetic anhydride and 5 ml. of pyridine. The solution was left at room temperature for 70 hr., then evaporated to dryness *in vacuo*. The residual oil was crystallized from a solution of isopropyl ether and acetone, m.p. 108–110°; $[\alpha]^{27}D$ +232° (*c* 0.5% in methanol); λ_{max}^{MeOH} 232 mμ (*a* 65.5), 290 mμ (*a* 2.6).

Anal. Calcd. for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.36; H, 7.59; N, 4.21.

Streptimidone Oxime.—The procedure of Siggia[6] was used to prepare the derivative. The titration mixture was extracted with ethyl ether and the extract was evaporated to dryness *in vacuo*. The residue was crystallized from 50% aqueous methanol, m.p. 141–146°; $[\alpha]^{27}D$ +65° (*c* 0.5% in methanol); λ_{max}^{MeOH} 233 mμ (*a* 79.8); λ_{max}^{KBr} 2.99, 5.74, 5.95, 6.01, 6.21, 7.83, 8.62, 9.24, 10.07, 10.60 and 11.02 μ.

Anal. Calcd. for $C_{16}H_{24}N_2O_4$: C, 62.31; H, 7.84; N, 9.09. Found: C, 62.45; H, 7.83; N, 9.25.

Dihydrostreptimidone.—Streptimidone (25 g.) was dissolved in 200 ml. of 95% ethanol and reduced catalytically with 2.5 g. of 5% palladium on calcium carbonate at room temperature at 20 p.s.i. The reaction stopped by itself after 1.05 moles of hydrogen had been taken up. The product was crystallized from an isopropyl ether–methanol solution; m.p. 47–49°; $[\alpha]^{26}D$ +80° (*c* 5% in methanol); λ_{max}^{MeOH} 289 mμ (*a* 0.54); Kuhn–Roth C-methyl, 2.16; *K. africana* activity, 6 units/mg.

Anal. Calcd. for $C_{16}H_{25}NO_4$: C, 65.06; H, 8.53; N, 4.74. Found: C, 65.05; H, 8.48; N, 4.83.

Tetrahydrostreptimidone.—Streptimidone (998 mg.) was dissolved in 25 ml. of 95% ethanol and reduced catalytically with 200 mg. of palladium on charcoal at room temperature and atmospheric pressure. After 3 hr. the hydrogen uptake was complete (1.99 moles). The reaction product was crystallized from isopropyl ether; m.p. 56.5–57°; $[\alpha]^{28}D$ +11° (*c* 5% in ethanol); λ_{max}^{MeOH} 286 mμ (*a* 0.14); *K. africana* activity, less than 2 units/mg.

Anal. Calcd. for $C_{16}H_{27}NO_4$: C, 64.62; H, 9.15; N, 4.71. Found: C, 64.65; H, 9.17; N, 4.77.

Tetrahydrostreptimidone acetate: m.p. 86.5–87°; $[\alpha]^{28}D$ +12° (*c* 5% in ethanol); λ_{max}^{MeOH} 285 mμ (*a* 0.16).

Anal. Calcd. for $C_{18}H_{29}NO_5$: C, 63.69; H, 8.61; N, 4.13. Found: C, 63.86; H, 8.86; N, 4.38.

Tetrahydrostreptimidone oxime: m.p. 159.5–162°; $[\alpha]^{25}D$ −21° (*c* 0.5% in methanol); ultraviolet spectrum, only end absorption.

Anal. Calcd. for $C_{16}H_{28}N_2O_4$: C, 61.51; H, 9.03; N, 8.97. Found: C, 61.79; H, 9.15; N, 8.83.

Hexahydrostreptimidone.—The crystalline streptimidone (970 mg.), dissolved in 25 ml. of acetic acid, was catalytically reduced with platinum at room temperature and atmospheric pressure. The reaction was complete when 2.9 moles of hydrogen had been taken up. Attempts to crystallize the product failed. The crude product melted at 95–102°; $[\alpha]^{28}D$ 0° (*c* 0.5% in methanol). The compound does not inhibit *K. africana*. $\lambda_{max}^{CHCl_3}$ 2.85, 2.90, 3.04, 3.34, 5.84, 6.83, 6.98, 7.23, 7.37, 7.96, 8.68 and 9.14 μ.

Anal. Calcd. for $C_{16}H_{29}NO_4$: C, 64.18; H, 9.76; N, 4.68. Found: C, 64.13; H, 9.78; N, 4.73.

Streptimidol.—One gram of sodium borohydride in 20 ml. of water and 20 ml. of 0.2 *M* phosphate buffer (*p*H 5.5) were added successively to 3.0 g. of streptimidone dissolved in 50% aqueous dioxane. The solution was kept at 8° for 48 hr., whereupon it was diluted with 100 ml. of water and extracted three times with 200-ml. portions of chloroform. The chloroform extracts were combined and evaporated to dryness *in vacuo;* residue, 0.74 g. of oil; λ_{max}^{MeOH} 230 mμ (*a* 80).

(12) R. B. Woodward, THIS JOURNAL, **63**, 1123 (1941); **64**, 72, 76 (1942).

(13) D. L. Kohberger, M. W. Fisher, M. M. Galbraith, A. B. Hillegas, P. E. Thompson and J. Ehrlich, paper in preparation.

Ozonolysis of Streptimidone.[14]—Ozone was passed through a solution of 1 g. of streptimidone in 20 ml. of methanol at −80° until the blue color of the ozone appeared (32 minutes). A standard thiosulfate control solution showed that two moles of ozone had reacted with the antibiotic. The ozonide was decomposed with aqueous ferrous sulfate and the volatile products were distilled into a solution of 2,4-dinitrophenylhydrazine hydrochloride in methanol. The resulting hydrazones were transferred to benzene solution and the latter solution was percolated through a column of deactivated aluminum oxide to remove the excess 2,4-dinitrophenylhydrazine. The benzene filtrate was evaporated to dryness *in vacuo* and the residue was chromatographed according to the procedure of Gordon, *et al.*,[15] using a mixture of silicic acid and Celite 545 (2:1). The 2,4-dinitrophenylhydrazones of methyl ethyl ketone (0.56 mole), formaldehyde (0.16 mole) and acetaldehyde (0.11 mole) were identified by their melting points, ultraviolet and infrared spectra, elemental analyses and paper chromatography, using *n*-heptane saturated with methanol.[16]

Alkaline Hydrolysis of Streptimidone.—Twenty grams of streptimidone was dissolved in 175 ml. of 1 *N* sodium hydroxide. The solution was left at room temperature for 2 hr., adjusted to *p*H 7, then extracted twice with 300-ml. portions of ethyl ether. The ether extract was dried with anhydrous sodium sulfate; the ether distilled off and the residue distilled *in vacuo*. A volatile oil, b.p. 45° at 2 mm., was obtained; λ_{max}^{EtOH} 232 mμ (*a* 123), 279 mμ (*a* 32.1); $\lambda_{max}^{CHCl_3}$ 5.80, 5.99, 6.13, 6.19, 6.84, 7.32, 7.93, 8.51, 9.41, 10.06, 11.02 and 11.45 μ.

A sample of the volatile oil was treated with 2,4-dinitrophenylhydrazine. The resulting hydrazones were first chromatographed on deactivated aluminum oxide from benzene solution to remove the unreacted 2,4-dinitrophenylhydrazine. The benzene filtrate was then chromatographed using a column of silica gel and bentonite (4:1)[17] prepared in benzene. A solution of 20% ethyl ether in benzene eluted a yellow compound, component B, which was crystallized from methanol, m.p. 113–114°; λ_{max}^{MeOH} 360 mμ (*a* 70.4) and at 230 mμ (*a* 102.5); $\lambda_{max}^{10\%\ CHCl_3-90\%\ EtOH-0.01\ N\ NaOH}$ 432 mμ.[18]

Anal. Calcd. for $C_{15}H_{18}N_4O_4$: C, 56.59; H, 5.70; N, 17.60. Found: C, 56.88; H, 5.65; N, 17.89.

Washing of the silica gel–bentonite column with 50% ethyl ether in benzene eluted a dark red hydrazone. The latter, component A, was crystallized from boiling ethyl acetate, m.p. 163–164°; λ_{max}^{EtOH} 382 mμ (*a* 89.1), 290 mμ (*a* 34.5), 259 mμ (*a* 44.1) and at 230 mμ (*a* 44.4); $\lambda_{max}^{10\%\ CHCl_3-90\%\ EtOH-0.01\ N\ NaOH}$ 460 mμ.

Anal. Calcd. for $C_{15}H_{18}N_4O_4$: C, 56.59; H, 5.70; N, 17.60. Found: C, 56.49; H, 5.80; N, 17.80.

The alkaline reaction mixture, after ether extraction of the volatile oil, was adjusted to *p*H 3.0 and the solution was extracted continuously with ethyl ether. Evaporation of the ether extract yielded an oil; an aliquot of the latter was chromatographed on Whatman #1 paper using a mixture of ethanol, water and concentrated ammonium hydroxide (16:3:1). The developed chromatograms were sprayed with brom phenol blue. Four zones were obtained: R_f 0.11, 0.22, 0.47 and 0.64. The same bands were obtained with the alkaline fraction from the hydrolysis of actidione.

Twenty grams of streptimidone was dissolved in 50 ml. of 6 *N* sodium hydroxide and heated on the steam-bath for 1 hr.; the solution was then placed in a semimicro steam distillation apparatus and steam distilled for 1.5 hr. The distillate was collected in chilled 0.5 *N* hydrochloric acid. The latter was extracted with ethyl ether to remove the volatile oil and the aqueous residue was evaporated to dryness *in vacuo*. The residue, in 0.5 ml. of water, was added to 7 ml. of a 5% aqueous solution of 4-hydroxyazobenzene-4′-sulfonic acid (*p*HABS). The resulting precipitate was centrifuged and recrystallized from water. The orange plates were identified as the ammonium salt of *p*-HABS; λ_{max} *p*H 7 phosphate buffer 353 mμ (*a* 76).

Anal. Calcd. for $C_{12}H_{13}SO_4N_3$: C, 48.80; H, 4.44; N, 14.23. Found: C, 48.93; H, 4.74; N, 14.33.

The ether extract, containing the volatile oil, was distilled to remove the ether and the residual oil was distilled *in vacuo;* b.p. 52–55° at 3 mm.; λ_{max}^{EtOH} 226 mμ (*a* 35), 279 mμ (*a* 97.2); $\lambda_{max}^{CHCl_3}$ 5.95, 6.10, 6.87, 7.25, 7.91, 8.96, 9.60, 9.90, 10.30 and 11.06 μ.

The 2,4-dinitrophenylhydrazone of the oil was formed and purified as described above in the hydrolysis of the antibiotic with 1 *N* sodium hydroxide. Only one hydrazone was obtained; it was identical with the red component A described above.

Treatment of the volatile oil (major maximum at 232 mμ), obtained from the hydrolysis of the antibiotic with 1 *N* sodium hydroxide, with 6 *N* sodium hydroxide at 100° for 35 minutes yielded an oil with a major maximum in the ultraviolet at 279 mμ and a minor at 226 mμ. The ultraviolet spectrum of the oil was identical with that obtained in the 6 *N* sodium hydroxide hydrolysis.

The original alkaline reaction mixture remaining in the distillation flask was chromatographed on paper using the ammoniacal aqueous ethanol system as described earlier. The same four brom phenol blue spots were obtained.

Alkaline Hydrolysis of Dihydrostreptimidone.—Dihydrostreptimidone (4.0 g.) was dissolved in 60 ml. of 1 *N* sodium hydroxide in a steam distillation apparatus and the solution was immediately steam distilled. The time of distillation was 0.5 hr. The distillate contained ammonia and a volatile oil which was transferred into ethyl ether. The extract was distilled to remove the ether and left an oil, 3,5-dimethyl-4-heptene-2-one, which was distilled; b.p. 166–168° at 745 mm.; λ_{max}^{EtOH} 281 (*a* 2.3); $n^{25}D$ 1.4382; $[\alpha]^{27}D$ −0.6° (*c* 5% in methanol); $\lambda_{max}^{CHCl_3}$ 5.81, 6.04, 6.83, 7.35, 8.04, 8.53, 8.95, 10.01, 10.46, 10.90, 11.08 and 11.58 μ.

2,4-Dinitrophenylhydrazone: m.p. 94–96°; λ_{max}^{EtOH} 360 mμ (*a* 68.5), 229 mμ (*a* 52.3).

Anal. Calcd. for $C_{15}H_{20}N_4O_4$: C, 56.24; H, 6.29; N, 17.49. Found: C, 56.28; H, 6.29; N, 17.76.

Ozonolysis of the volatile oil in carbon tetrachloride solution and subsequent decomposition of the ozonide with aqueous ferrous sulfate gave methyl ethyl ketone which was isolated as its 2,4-dinitrophenylhydrazone; m.p. 113–114°; λ_{max}^{EtOH} 360 mμ (*a* 87.4).

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.92; H, 4.78; N, 22.18.

Treatment of the volatile oil from dihydrostreptimidone with 1 *N* methanolic sodium hydroxide for 24 hr. at 100° shifted the maximum of the oil to 230 mμ.

Alkaline Hydrolysis of Tetrahydrostreptimidone.—Eleven grams of tetrahydrostreptimidone was dissolved in 50 ml. of 3 *N* sodium hydroxide and the solution was steam distilled for 1.5 hr. The distillate, containing ammonia and the volatile oil, was adjusted to *p*H 7 and extracted with ethyl ether (three times with 300 ml.). The ether extract was dried with anhydrous sodium sulfate; the ether was removed at atmospheric pressure and the residual oil was distilled; yield 3.46 g. of 3,5-dimethyl-2-heptanone; b.p. 174–175°; $n^{25}D$ 1.4187; $[\alpha]^{27}D$ −0.7° (*c* 5% in methanol); λ_{max}^{MeOH} 282 mμ (*a* 0.24); $\lambda_{max}^{CHCl_3}$ 5.83, 6.83, 7.25, 7.35, 8.08, 8.45, 8.70, 9.00, 10.35 and 10.45 μ.

Anal. Calcd. for $C_9H_{18}O$: C, 76.00; H, 12.76. Found: C, 76.29; H, 12.94.

2,4-Dinitrophenylhydrazone: m.p. 72–73°; λ_{max}^{EtOH} 360 mμ (*a* 69.4), 228 mμ (*a* 52.3).

Anal. Calcd. for $C_{15}H_{22}N_4O_4$: C, 55.88; H, 6.88; N, 17.38. Found: C, 55.52; H, 6.73; N, 17.43.

Semicarbazone: m.p. 104–105°; λ_{max}^{EtOH} 226 mμ (*a* 70.4).

Anal. Calcd. for $C_{10}H_{21}N_3O$: C, 60.26; H, 10.62; N, 21.09. Found: C, 60.28; H, 10.44; N, 21.01.

The residual alkaline solution in the distillation unit was chromatographed on paper using ammoniacal aqueous ethanol as described previously. Four brom phenol blue spots were obtained which were identical with those obtained from the corresponding acid fraction of actidione.

L-Ethyl-2,4-dimethyl-2-carbethoxyhexanoate (VII).—The bromination of 2-methylbutanol ($\alpha^{28}D$ −9.32° [l = 2]) to L-1-bromo-2-methylbutane was accomplished according to

(14) Ozonolysis experiments were completed with the coöperation of Drs. J. P. Kutney, Calvin L. Stevens and Carl Djerassi of Wayne State University, Detroit, Mich.

(15) B. E. Gordon, F. Wopat, Jr., H. D. Burnham and L. C. Jones, Jr., *Anal. Chem.*, **23**, 1754 (1951).

(16) D. F. Meigh, *Nature*, **170**, 579 (1952).

(17) L. I. Braddock, K. Y. Garlow, L. I. Grim, A. F. Kirkpatrick, S. W. Pease, A. J. Pollard, E. F. Price, T. I. Reissmann, H. A. Rose and M. L. Willard, *Anal. Chem.*, **25**, 301 (1953).

(18) C. J. Timmins, *J. Chem. Soc.*, 2613 (1957).

the method of Crombie and Harper[19] using phosphorus tribromide and pyridine; yield 50%; b.p. 118–120°; $n^{25}D$ 1.4425; $\alpha^{28}D$ +4.69 (l = 1).[20]

An ethanol solution of methyl diethylmalonate (7.3 g., 0.042 mole) was added to a solution of 0.9 g. (0.04 mole) of sodium in 30 ml. of absolute ethanol, and the solution was refluxed for 40 minutes; 5.38 g. (0.0356 mole) of L-1-bromo-2-methylbutane in ethanol solution was slowly added (20 minutes) to the above. The reaction mixture was refluxed for 3 hr., allowed to stand at room temperature overnight and then concentrated *in vacuo*. The residue was treated with water and then extracted three times with ether. The combined ether layers were extracted with 0.5 *N* hydrochloric acid and finally with water. The organic layer was dried over sodium sulfate and then evaporated to a residual oil. Distillation afforded two fractions: (a) 3 g. of methyl diethylmalonate, b.p. 88–92° (14 mm.) and (b) 3.8 g. (43%) of the expected disubstituted malonic ester VII, b.p. 120–123° (14 mm.); $n^{25}D$ 1.4270; $[\alpha]^{28}D$ +7.7° (*c* 2.1% in chloroform).

Anal. Calcd. for $C_{13}H_{24}O_4$: C, 63.90; H, 9.90. Found: C, 63.98; H, 9.94.

2,4-Dimethylhexanoic Acid (VIII).[21]—A sample (10.5 g.) of VII was added to a solution of 11 g. of potassium hydroxide, 30 ml. of water and 100 ml. of methanol. This mixture was heated under reflux for 20 hr. and then concentrated under reduced pressure to about 30 ml. Acidification of this solution with hydrochloric acid precipitated an oil which was extracted into ether. The ether extract was dried over sodium sulfate and then concentrated to a residual oil. Decarboxylation of the malonic acid proceeded smoothly at 155–160°. After 2 hr. at this temperature, distillation afforded 5.0 g. (81%) of 2,4-dimethylhexanoic acid (VIII), b.p. 113–116° (18 mm.).

3,5-Dimethyl-2-heptanone (VI).—Acid VIII (4.0 g.) was refluxed in benzene solution for 3 hr. with 5.2 ml. of freshly distilled thionyl chloride. Concentration and distillation of the reaction mixture gave 3.6 g. of the corresponding acid chloride as a colorless liquid, b.p. 57–59° (8 mm.). An anhydrous benzene solution of this material was added to a warm benzene slurry of dimethylcadmium prepared by the method of Cason[22] starting with 2.13 g. (0.089 mole) of magnesium. The reaction mixture was heated under reflux for 15 minutes and then allowed to stand for an additional 10 minutes. The mixture was well cooled in an ice-bath and carefully treated with dilute sulfuric acid. After complete hydrolysis, the layers were separated and the organic layer washed with 5% sodium carbonate and then with water. The organic layer was cautiously concentrated to a residual oil which was then dissolved in a solution of 0.5 *N* sodium hydroxide in 50% aqueous ethanol. This mixture was allowed to stand overnight and was then heated on the steam-bath for 90 minutes. Water was added and the diluted mixture then extracted three times with ether. The combined organic extracts were dried over sodium sulfate and distilled to remove the ether. The residual oil then distilled as a single portion, b.p. 168–172°, which afforded 1.27 g. of the colorless ketone VI possessing the same pleasant odor as the ketone isolated *via* basic treatment of tetrahydrostreptimidone. The infrared of this material as a liquid film or in chloroform, except for slight intensity differences, was identical with the corresponding ketone from tetrahydrostreptimidone, $n^{25}D$ 1.4178; $[\alpha]^{27}D$ +7.9° (*c* 5.5% in methanol).

Anal. Calcd. for $C_9H_{18}O$: C, 76.00; H, 12.76. Found: C, 75.63; H, 12.52.

2,4-Dinitrophenylhydrazone.—A solution of 120 mg. (0.86 mmole) of VI in 5 ml. of ethanol was treated with a solution of 215 mg. (1.09 mmoles) of 2,4-dinitrophenylhydrazine in 70 ml. of 1.7 *N* hydrochloric acid. An immediate yellow oil formed, and after standing 24 hours the mixture was extracted twice with petroleum ether. The dried organic layer was evaporated to about 5 ml. and the remaining solution was chromatographed on a column of 12 g. of acid-washed alumina. The derivative was eluted with 1:1 petroleum ether:benzene and recrystallized several times from petroleum ether (b.p. 30–60°) to yield the 2,4-dinitrophenylhydrazone, m.p. 53–55°. Further recrystallization did not appreciably raise the melting point (m.p. 54–56°); a mixed melting point with the 2,4-dinitrophenylhydrazone of the ketone from tetrahydrostreptimidone exhibited the intermediary range of 55–67°; λ_{max}^{EtOH} 360 mμ (*a* 67.0), 228 mμ (*a* 59.5). The infrared spectrum of this derivative was identical with the corresponding derivative from tetrahydrostreptimidone.

Anal. Calcd. for $C_{15}H_{22}N_4O_4$: C, 55.88; H, 6.88; N, 17.38. Found: C, 55.50; H, 6.88; N, 17.09.

Semicarbazone.—This derivative was prepared in the usual manner and showed a melting range of 79–85° after repeated crystallization from aqueous ethanol; λ_{max}^{EtOH} 225 mμ (*a* 70.2); the "finger print" region of the infrared spectrum was nearly superimposable with that of the semicarbazone from the ketone derived from tetrahydrostreptimidone.

Anal. Calcd. for $C_{10}H_{21}N_3O$: C, 60.26; H, 10.62; N, 21.09. Found: C, 60.27; H, 10.57; N, 21.25.

Dehydration of Tetrahydrostreptimidone.—Tetrahydrostreptimidone (2 g.), dissolved in 60 ml. of dry benzene, was treated with 4 g. of phosphorus pentoxide. The mixture was refluxed for 15 minutes, chilled and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was crystallized from isopropyl ether. The yield was 627 mg.; m.p. 78–80°; $[\alpha]^{27}D$ +7.2° (*c* 5% in ethanol); *pK'*a, 11.5 (50% aqueous methanol); λ_{max}^{EtOH} 225 mμ (*a* 44.5), 322 mμ (*a* 0.2); λ_{max}^{KBr} 5.76, 5.85, 6.00, 6.12 and 10.05 μ.

Anal. Calcd. for $C_{16}H_{25}NO_3$: C, 68.78; H, 9.02; N, 5.01. Found: C, 68.63; H, 9.29; N, 5.05.

Reaction of Tetrahydrostreptimidone and Benzylamine.—Three grams of tetrahydrostreptimidone was heated on the steam-bath with 7.5 ml. of benzylamine for 2 hr. and the solution was chilled overnight at 5°. The precipitated solid was centrifuged, washed with cold chloroform and crystallized three times from 10 ml. of boiling methanol; m.p. 170–172°; $\lambda_{max}^{90\%\ EtOH}$ 267 mμ (*a* 0.52), 263 mμ (*a* 0.86), 258 mμ (*a* 1.13), 252 mμ (*a* 0.91), 248 mμ (*a* 0.67).

Anal. Calcd. for $C_{21}H_{25}N_3O_2$: C, 71.77; H, 7.17; N, 11.96. Found: C, 71.67; H, 7.05; N, 11.99.

This product was compared with the compound which we obtained by reacting actidione and benzylamine according to the method of Kornfeld, *et al.*[1] The actidione degradation product melted at 170–172° and gave no depression in melting point when mixed with the degradation product from streptimidone.

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DETROIT, MICHIGAN

(19) L. Crombie and S. H. Harper, *J. Chem. Soc.*, 2685 (1950); these authors review the previous literature concerning optically pure 2-methylbutanol. These data show that our starting alcohol was at least 97% optically pure 2-methylbutanol.

(20) According to L. Crombie, M. Manzoor-i-Khuda and R. J. D. Smith, *ibid.*, 484 (1957), this rotation of L-1-bromo-2-methylbutane indicates an optical purity of 99%.

(21) An alternate synthesis of this acid has recently been reported by H. Stetter and U. Milbers, *Chem. Ber.*, **91**, 374 (1958).

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