256. The Absolute Configuration of the Glutarimide Antibiotics Streptimidone and 9-Methylstreptimidone[1])

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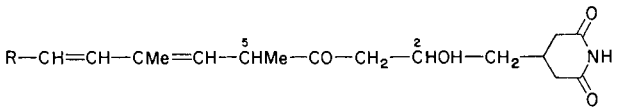
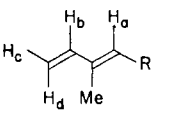
Summary. Chemical and spectroscopic studies have established the absolute configuration of the glutarimide antibiotics streptimidone and 9-methylstreptimidone, which can now be represented as the (2*R*,5*S*,6*E*)-structures **14** and **15**, respectively.

1. Introduction. – The glutarimide group of antibiotics [1] comprises some twenty compounds characterized structurally by the presence of a glutarimide ring bearing a side chain at the 3-position. In most members the side chain terminates in a substituted carbocyclic ring, usually cyclohexyl but occasionally phenyl, although in three representatives, streptimidone (**1**) [2], protomycin [3], and the recently described 9-methylstreptimidone (**2**) [4] [5], the side chain is acyclic. The biogenesis of these antibiotics proceeds basically in polyketide fashion, with notable features including initiation of the carbon skeleton by a malonate (or malonamate) unit, the presence of a branch completing the glutarimide ring, and the introduction of additional methyl substituents [5] [6]. Whilst the configuration of the cyclohexyl compounds is firmly established [1], little is known about the configuration of the three non-carbocyclic members. In view of the significant biological activity of streptimidone (**1**) against yeasts, fungi, and protozoa [7], and of 9-methylstreptimidone (**2**) against yeasts, fungi, and notably viruses [4] [5], we here define the absolute configuration of these two homologous antibiotics. Such information is necessary for a complete understanding of the mode of action of these compounds, which in eukaryotic cells involves the inhibition of protein synthesis [8].

2. Results and Discussion. – The stereochemical problems presented by streptimidone (**1**) and 9-methylstreptimidone (**2**) concern the 6,8-diene systems and the two tetrahedral chiral centres at C(2) and C(5).



1: R = H ; **2**: R = Me



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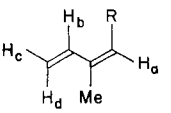

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3, 4: R = alkyl

[1]) This work was presented in part at the National Conference of the Organic Division of the Royal Australian Chemical Institute, held in Brisbane, August 1975.

2.1 *Configuration of the 6,8-Diene Systems.* In both streptimidone (**1**) and 9-methylstreptimidone (**2**) [5] the configuration of the C(6), C(7) double bond cannot be unambiguously defined by the allylic coupling constant ($J = 1.2$ and 1.0 Hz, respectively) between the H_3C–C(7) and H–C(6) [9]. In neither compound is there a detectable nuclear *Overhauser* enhancement [10] in the intensity of the H–C(6) resonance upon irradiation of the H_3C–C(7), suggesting that the C(6),C(7) double bond has (*E*) configuration in each case [5]. For streptimidone itself, this assignment was confirmed by comparison of chemical shift values with those of known model 1,3-dienes. In Table 1 are recorded the chemical shifts of the olefinic protons H_a, H_b, H_c, and H_d of 1,2-disubstituted 1,3-dienes, the ranges shown for (*E*)-**3** and (*Z*)-**4** being derived from seven compounds measured in carbon tetrachloride by *Carman* [11]. The critical proton which permits a clear distinction between the (*E*) and (*Z*) configuration is H_b, which in the case of streptimidone (**1**) in the same solvent resonates at δ 6.30 (*cf.* Table 1) and defines the C(6), C(7) double bond configuration in that compound as (*E*).

Table 1. *Olefinic Proton Resonances*[a]) *of 1,3-Dienes*

1,3-Diene	Solvent	H_a	H_b	H_c	H_d
(*E*)-**3**	CCl_4	5.39–5.42	6.28–6.31	4.85–4.88	5.00–5.02
(*Z*)-**4**	CCl_4	5.20–5.45	6.65–6.85	4.97–5.03	5.04–5.11
Streptimidone (**1**)	CCl_4	5.33	6.30	4.97	5.11
Streptimidone (**1**)	$CDCl_3$	5.32	6.35	5.04	5.18
Streptimidol H	$CDCl_3$	5.30	6.39	5.02	5.16
Streptimidol L	$CDCl_3$	5.26	6.34	4.99	5.13
9-Methylstreptimidone (**2**)	$CDCl_3$	5.17	5.83	5.50	–

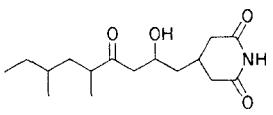
[a]) Chemical shifts as δ values in ppm relative to internal TMS.

Streptomidone (**1**) is a β,γ-, δ,ε-unsaturated dienone in which the π electrons of the diene interact with the non-bonding electrons of the ketone, resulting in enhanced intensity (ε 790 [2]) of the carbonyl $n \rightarrow \pi^*$ UV. absorption at 291 nm as expected [12]. In case this interaction is influencing chemical shift values in the diene and thus invalidating the above stereochemical conclusions, streptimidone (**1**) was reduced with sodium borohydride, in buffered solution to prevent conjugation prior to reduction. The resulting diastereoisomeric streptimidols H (higher Rf) and L (lower Rf), which were not separated by previous workers [2], each showed olefinic proton resonances in chloroform closely similar in chemical shift to those of streptimidone in the same solvent (*cf.* Table 1). Thus the electronic interaction between the chromophores in streptimidone (**1**) is not significantly affecting chemical shift values in the 1,3-diene system, the (*E*) configuration of which is therefore confirmed.

Unfortunately a similar chemical shift analysis cannot be applied to the C(6),C(7) double bond of 9-methylstreptimidone (**2**). As expected [13], in this case the additional methyl substituent itself alters chemical shifts in the diene considerably (*cf.* Table 1) and the chemical shifts which have been assigned in known 1,2,4-trisubstituted 1,3-dienes [14] are not distinctive for (1*E*) or (1*Z*) configuration. However, if the complete correspondence of configuration (defined below) between the tetrahedral chiral

centres in streptimidone (**1**) and its homologue **2** extends also to the diene systems, then 9-methylstreptimidone (**2**) would be predicted to have the (6-*E*) configuration. This configuration is indicated both by the nuclear *Overhauser* experiment and the allylic coupling constant of 1.0 Hz (*cf.* [14]) described above. The disubstituted C(8),C(9) double bond in 9-methylstreptimidone (**2**) has been shown previously to have the (*Z*) configuration [5].

2.2. *Configuration at C(5).* The major contribution to the chiroptical properties of the antibiotics **1** and **2** will arise from the interacting orbitals [12] in the β,γ-, δ,ε-dienone chromophore, since the glutarimide system, although chromophoric [15], is in each case remote from any chirality centre. Furthermore, the contribution of chirality at C(2), which is β to the carbonyl group, will be minor relative to the dominant contribution of chirality at C(5) encompassed by the dienone system itself [12]. Thus, since the ORD. and CD. curves of streptimidone (**1**) and 9-methylstreptimidone (**2**) exhibit very similar multiple *Cotton* effects, we can conclude that the two compounds have the same absolute configuration at least at C(5). Since model β,γ-, δ,ε-dienones of known absolute configuration were lacking, this C(5) configuration was defined by degradation of the more readily available antibiotic streptimidone.



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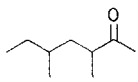

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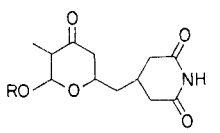
Streptimidone (**1**) was hydrogenated catalytically to the tetrahydro derivative **5** which was subjected to pyrolysis in vacuum. The resulting ketone **6** was identified directly by mass spectrometry, and by both mass and ^{1}H-NMR. spectra after conversion to its 2,4-dinitrophenylhydrazone derivative. The pyrolytic *retro*-aldol conditions were chosen in order to avoid racemization of the centre α to the ketone group in either **5** or **6**, which might occur under basic catalysis in solution [16]. Both ketones **5** and **6** would be expected to be mixtures of diastereoisomers epimeric at C(7) and C(5), respectively, as the result of non-stereospecific hydrogenation of the diene **1**. However, these centres in each ease are γ to the carbonyl group, the principal chiral chromophore, and consequently would exert only minor influence on its chiroptical properties in comparison to asymmetry at the α-position [17]. Similar considerations apply to the influence of the hydroxylated β-centre in **5**, and here also the glutarimide chromophore is remote from any chirality centre. Consequently the chiroptical properties of **5** and, more particularly, of **6** would be dominated by asymmetry at the α-position. Since both ketones **5** and **6** show single positive *Cotton* effects in both ORD. and CD. which match the positive effect of (3*S*)-3-methyl-2-pentanone [17], they can be assigned *S* configuration at C(5) and C(3), respectively. The parent antibiotic **1**, and its homologue 9-methylstreptimidone (**2**) thus both have the *S* configuration at C(5).

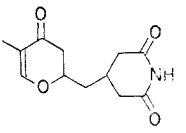
2.3. *Configuration at C(2).* Our initial approach towards definition of the absolute configuration at the secondary alcohol centre involved *Horeau*'s direct method [18], which has the advantage that no degradation is necessary. The method depends upon

the partial kinetic resolution of racemic 2-phenylbutyric anhydride used to acylate the alcohol, residual anhydride being analysed by gas chromatography of the diastereoisomeric amides formed by reaction with (*R*)-1-phenylethylamine [19]. In our hands the reference alcohols L-menthol and quinine gave results in agreement with [19]. However, when the sequence was applied to streptimidone (**1**), 9-methylstreptimidone (**2**) and the related glutarimide antibiotic cycloheximide of established *R* configuration at the hydroxylated centre (C(2)) [1], the extent of resolution of the anhydride was low. Assuming the glutarimide residue to be effectively larger than the dienone system in **1** and **2**, but smaller than the dimethylcyclohexanone residue in cycloheximide, then the enantiomeric yields consistently indicated the *R* configuration of the hydroxylated C(2) in each case. The insensitivity of the *Horeau* method when applied to the dienones **1** and **2** is probably due to the fact that the 'medium' and 'large' groups are each separated by a methylene group from the chiral hydroxylated centres [18] [20].

Since this direct approach was not sufficiently conclusive, we turned to chiroptical studies of degradation products in which the asymmetry at C(5) had been destroyed. Ozonolysis of streptimidone (**1**) in methanol gave an unresolved mixture, which was suggested by ^{1}H-NMR. spectroscopy to contain diastereoisomeric hemiacetal derivatives **7** derived from the initially formed δ-hydroxy-aldehyde. Acid treatment of this mixture yielded the known 3-methyl-5,6-dihydro-4-pyrone derivative **8** [6]. An identical compound **8**, in particular as regards ORD. and CD. properties, was obtained also from 9-methylstreptimidone (**2**)[2]), thus establishing that the two antibiotics have the same absolute configuration at C(2).



7: R = H, OH, Me or OMe



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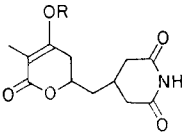
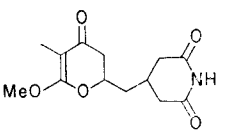


9: R = H; **10**: R = Me



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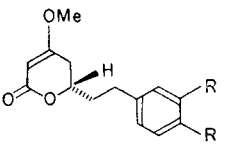


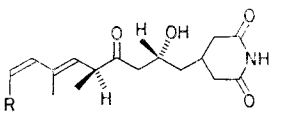
12: R = H; **13**: R, R = OCH_2O

[2]) This constitutes the first chemical correlation of the antibiotics **1** and **2**, and provides direct, non-spectroscopic evidence for the presence of a 3-substituted glutarimide nucleus in **2**.

The mixture of hemiacetal derivatives **7** from each antibiotic, on oxidation in acidic aqueous media, afforded the 4-hydroxy-3-methyl-5,6-dihydro-2-pyrone **9**. The same enantiomer of **9** was obtained from both streptimidone and 9-methylstreptimidone. The structure of this 5,6-dihydro-2-pyrone follows from its MS., its NMR. characteristics, and particularly its UV. absorption (λ_{max} = 248 nm in acidic ethanol changing to λ_{max} = 280 nm upon the addition of base), which is characteristic of an ionizable β-hydroxy-α,β-unsaturated ester [21]. This UV. spectrum specifically establishes the 4-hydroxy-2-pyrone form **9** as the predominant tautomer of the pyrone present in acidic ethanol. Consequently, its negative *Cotton* effect visible in this solvent in both ORD. and CD., when compared with the positive *Cotton* effect CD. curves of (6*S*)-dihydrokawain (**12**) and (6*S*)-dihydromethysticin (**13**) [22], establishes the *R* configuration at C(6) in the 5,6-dihydro-2-pyrone **9**. It follows that streptimidone (**1**) and 9-methylstreptimidone (**2**) have the same *R* configuration at C(2).

These conclusions based on the potentially tautomeric 4-hydroxy-2-pyrone **9** were confirmed by study of its two *O*-methylated derivatives **10** and **11**, where tautomerism is no longer possible. Prepared with diazomethane, these isomers were readily distinguished and identified as the 4-methoxy-5,6-dihydro-2-pyrone **10** and the 2-methoxy-5,6-dihydro-4-pyrone **11** by their UV. maxima at 252 and 276 nm, respectively [23]. As expected, the ORD. and CD. characteristics of **10** matched those of the free 4-hydroxy tautomer **9**.

These optical correlations depend upon the preferred conformations of the pyrone rings in **9** and **10** being those in which the large substituent at C(6) is equatorial in each case [24]. This would be the expected conformation, and is established by the 30 Hz band width of the signal of H–C(6) in the NMR. spectra of these compounds, which can only result from a quasi-axially oriented proton. The configurational assignments for the 2-pyrones **9** and **10** are also in accordance with the semi-empirical helicity rule for the n $\rightarrow \pi^*$ transition of the carbonyl group in unsaturated lactones [25].



14: R = H ; **15**: R = Me

3. Conclusion. – The present work demonstrates the complete correspondence of configuration between the two non-carbocyclic glutarimide antibiotics streptimidone and 9-methylstreptimidone, which can now be represented as the (2*R*, 5*S*, 6*E*)-structures **14** and **15**, respectively. The hydroxylated centre C(2) in these compounds has the same configuration as that found to date in carbocyclic members of the glutarimide group [1].

We are indebted to Dr. *L. Coronelli*, Gruppo Lepetit s.p.a., Milan, for a culture of *Streptomyces* sp. E/887 used to produce 9-methylstreptimidone. We thank Dr. *J. E. Fildes* for microanalyses, Mr. *C. Arandjelovic* and Mr. *B. Paal* for NMR. spectra, and Mr. *M. Chapman* for MS. data.

Experimental

General. Melting points were determined on a *Kofler* stage and are uncorrected. Preparative TLC. was run on methanol-washed silica gel. UV., ORD. and CD. spectra are specified in λ_{max} (ε), λ_{max} ([Φ]) and λ_{max} ($\Delta\varepsilon$), respectively; concentrations (c) are given in mg/ml, λ_{max} in nm. NMR. spectra are described by chemical shifts (δ) and coupling constants J (in Hz) for solutions with tetramethylsilane as internal reference ($\delta = 0$ ppm). Instruments used: UV., *Unicam* SP 800; ORD. and CD., *Jasco* ORD/UV-5; IR., *Perkin-Elmer* 257 (absorptions given in cm^{-1}); NMR., *Varian* HA-100; MS., *GEC-AEI* MS 902; GLC.-MS., *Varian* Mat 111 with 180 cm × 3 mm i.d. glass column containing 2% OV-17; GLC., *Perkin-Elmer* 900 with 180 cm × 2 mm i.d. glass column containing 2% OV-17 operating at 175° isothermally.

Streptimidone (**1**). Streptimidone was obtained from *Calbiochem* and *Parke, Davis and Co.* If necessary it was purified by preparative TLC. in 1-propanol/chloroform 8:92, or by extraction with ethyl acetate at room temp. which separated insoluble decomposition products. – ORD. ($c = 0.13$ and 2.8, MeOH): 270 (– 27280), 292 (0), 313 (+23300), 350 (+6120), 589 (+535), 610 (+480). – CD. ($c = 0.008$, MeOH): 215 (0), 235 (– 79.1), 255 (0), 291 (+79.1), 330 (0).

9-Methylstreptimidone (**2**). 9-Methylstreptimidone was produced by fermentation and purified as described in [5] – ORD. ($c = 0.20$, 1.1 and 3.5, MeOH): 258 (– 24000), 293 (0), 313 (+13400), 350 (+3160), 589 (+290), 610 (+280). – CD. ($c = 0.016$, MeOH): 220 (– 11.7), 239 (– 43.3), 260 (0), 291 (+43.9), 330 (0).

Reduction of streptimidone with sodium borohydride. To streptimidone (80 mg) in ethanol/water 3:1 (50 ml) containing bromocresol green indicator was added dropwise with ice-cooling and stirring sodium borohydride (65 mg) in 1.3 ml of 0.1 N aqueous sodium hydroxide. The pH of the mixture was maintained acidic by repeated dropwise addition of 2 N aqueous acetic acid. After destruction of the excess of sodium borohydride with acetone, 50 ml of water were added, and the solution was saturated with sodium chloride and extracted with ethyl acetate. The extracts were dried, evaporated, and subjected to prep. TLC. in chloroform/methanol 9:1. Two isomers of streptimidol were isolated.

The isomer with the higher Rf value was designated *streptimidol H* (8.6 mg), m.p. 107–109°. – UV. (MeOH): 230. – IR. ($CHCl_3$): 3480 and 3380 (OH, NH), 1735 and 1710 (glutarimide CO). – NMR. ($CDCl_3$): 8.06 (*s*, N–H); 6.39 (*d* × *d*, $J_{trans} = 18$, $J_{cis} = 11$, H–C(8)); 5.30 (*d*, $J = 9.5$, H–C(6)); 5.16 (*d*, $J_{trans} = 18$, H–C(9)); 5.02 (*d*, $J_{cis} = 11$, H–C(9)); 3.5–4.1 (*m*, H–C(2) and H–C(4)); 1.81 (*s*, H_3C–C(7)); 1.01 (*d*, $J = 7$, H_3C–C(5)). – MS. (*m/e*): 277.1674 ($M^+ - H_2O$, calc. for $C_{16}H_{23}NO_3$ 277.1677), 200.0921 ($M^+ - C_7H_{11}$, calc. for $C_9H_{14}NO_4$ 200.0922), 182 ($C_9H_{12}NO_3$), 165 ($C_9H_{11}NO_2$), 96.0937 (calc. for C_7H_{12} 96.0938).

The isomer with the lower Rf value was designated *streptimidol L* (18.2 mg), m.p. 97–100°. – UV. (MeOH): 230 (23500). – IR. ($CHCl_3$): 3500 and 3380 (OH, NH), 1735 and 1710 (glutarimide CO). – NMR. ($CDCl_3$): 8.12 (*s*, N–H); 6.34 (*d* × *d*, $J_{trans} = 18$, $J_{cis} = 11$, H–C(8)); 5.26 (*d*, $J = 9.5$, H–C(6)); 5.13 (*d*, $J_{trans} = 18$, H–C(9)); 4.99 (*d*, $J_{cis} = 11$, H–C(9)); 3.6–4.15 (*m*, H–C(2) and H–C(4)); 1.80 (*s*, H_3C–C(7)); 1.08 (*d*, $J = 7$, H_3C–C(5)). – MS. (*m/e*): 277.1672 ($M^+ - H_2O$, calc. for $C_{16}H_{23}NO_3$ 277.1677), 200.0924 ($M^+ - C_7H_{11}$, calc. for $C_9H_{14}NO_4$ 200.0922), 182 ($C_9H_{12}NO_3$), 165 ($C_9H_{11}NO_2$), 96.0939 (calc. for C_7H_{12} 96.0939).

6,7,8,9-Tetrahydrostreptimidone (**5**). Streptimidone (82 mg) in ethanol (3.5 ml) was hydrogenated over 10% Pd/C (20 mg) at room temp. and pressure for 18 h, following the method of *Frohardt et al.* [2]. The filtered solution was evaporated and the residue extracted with ethyl acetate. Purification by preparative TLC. in 1-propanol/chloroform 8:92 gave tetrahydrostreptimidone (**5**) as a mixture of diastereoisomers (53 mg), m.p. 52–54°, $[\alpha]_D^{26} = +10°$ ($c = 0.56\%$, MeOH). *Frohardt et al.* [2] report m.p. 56.5–57°, $[\alpha]_D^{28} = +11°$ ($c = 5\%$, EtOH). – UV. (MeOH): 227 inflect. (280), 237 inflect. (180), 251 inflect. (105), 282 (60). – ORD. ($c = 2.66$ and 4.08, MeOH): 245 (– 701), 263 (– 1115), 282 (0), 298 (+745), 400 (+70), 589 (+30). – CD. ($c = 2.66$, MeOH): 238 (– 0.13), 257 (0), 280 (+0.38), 309 (0). – IR. (CCl_4): 3370 (OH, NH), 1740 and 1720 (glutarimide CO, ketone CO). – NMR. ($CDCl_3$): 8.55 (*s*, N–H); 4.15 (*m*, H–C(2)); 3.50 (*s*, O–H); 1.08 and 1.06 (*d*, $J = 7$, and *d*, $J = 7$, H_3C–C(5) of the two diastereoisomers), 0.89 (*m*, H_3C–C(7) and H_3C–C(8) of the two diastereoisomers). – MS. (*m/e*): 279 ($M^+ - H_2O$), 227 ($M^+ - C_5H_{10}$), 209 ($M^+ - H_2O - C_5H_{10}$), 198 ($M^+ - C_7H_{15}$), 180 ($M^+ - H_2O - C_7H_{15}$), 127 ($C_8H_{15}O$).

Retro-*aldol cleavage of 6,7,8,9-tetrahydrostreptimidone* (**5**). Tetrahydrostreptimidone **5** (30 mg) in a closed sublimation apparatus was heated from 185° to 235° during 20 min and then held at 235° for 10 min. The sublimate, condensed on a cold finger at – 77°, was collected with methanol and the solution distilled twice at 1.0 mm. GLC.-MS. analysis of the methanol solution showed the presence of 3,5-dimethylheptan-2-one (**6**). – MS. (*m/e*): 99 ($M^+ - CH_3CO$), 85 ($M^+ - C_4H_9$), 72 ($M^+ - C_5H_{10}$), 57 (C_4H_9), 43 (CH_3CO). – UV. (MeOH): 275. – ORD. (MeOH): 258 (negative), 280 (0), 296 (peak). – CD. (MeOH): 230 (0), 275 (positive maximum), 310 (0).

To the methanol solution of the ketone **6** was added 2,4-dinitrophenylhydrazine in 2 N sulfuric acid. After evaporation of the methanol, the residual aqueous solution was extracted with benzene. Preparative TLC. in chloroform of the dried extract yielded the 2,4-dinitrophenylhydrazone of the ketone **6** (1.5 mg). – UV. (EtOH): 229, 260 inflect., 362. – NMR. ($CDCl_3$): 9.12 (*d*, $J_{meta} = 1.2$, H–C(3′)); 8.30 (*d* × *d*, $J_{meta} = 1.2$, $J_{ortho} = 5.0$, H–C(5′)); 7.93 (*d*, $J_{ortho} = 5.0$, H–C(6′)); 2.65 (*m*, $J = 7$, H–C(3)); 2.00 and 1.98 (*s* and *s*, 3H–C(1) of the (*Z*) and (*E*) isomer); 1.14 (*d*, $J = 7$, H_3C–C(3)); 0.90 (*m*, H_3C–C(5) and H_3C–C(6)). – MS. (*m/e*): 322.1644 (M^+, calc. for $C_{15}H_{22}N_4O_4$ 322.1641), 307 ($M^+ - CH_3$), 265 ($M^+ - C_4H_9$), 252.0856 ($M^+ - C_5H_{10}$, calc. for $C_{10}H_{12}N_4O_4$ 252.0858), 219.0518 (calc. for $C_9H_7N_4O_3$ 219.0518), 217.0726 (calc. for $C_{10}H_9N_4O_2$ 217.0725), 192.0648 (calc. for $C_8H_8N_4O_2$ 192.0647).

Direct analysis of secondary alcohol configurations. The secondary alcohols (10 μmol) were reacted with racemic 2-phenylbutyric anhydride, the excess of anhydride was reacted with (*R*)-1-phenylethylamine, and the resulting (*R*)-1-phenylethylamides were analysed by GLC. according to the method of *Gilbert* & *Brooks* [20]. Peak increments as defined [20] for the amide of the (*R*)-acid, the configurational type [20] indicated by this result, and the resulting absolute configurational assignments are given for the various alcohols used: (*R*)-menthol, – 4.9, I, *R*; (*S*)-quinine, + 9.7, II, *S*; (2*R*)-cycloheximide, – 1.6, I, *R*; streptimidone, + 1.3, II, *R*; 9-methylstreptimidone, + 0.8, II, *R*.

The 3-methyl-5,6-dihydro-4-pyrone **8**. Streptimidone (150 mg) in methanol (3 ml) was ozonized to completion at – 78°, and the solvent was evaporated. The residue on TLC. showed a major zone which gave a positive test for peroxides, and probably contained the unresolved hemiaceta. derivatives **7**. This mixture was largely unaltered by reduction with zinc and acetic acid. – NMRl ($CDCl_3$): H–C=O absent, 5.6–5.0 (several *d* and *m* resonances, H–C(2)).

Without prior purification the residue was dissolved in benzene (10 ml) and 2-butanol (4 ml) containing *p*-toluenesulfonic acid (5 mg). After refluxing for 2 h in a *Soxhlet* apparatus containing molecular sieves (*Linde* type 4A), the concentrated solution was chromatographed on silica gel in chloroform/methanol, and then again on silica gel in chloroform, to give the *3-methyl-5,6-dihydro-4-pyrone* **8** (20 mg), m.p. 201–202° (with change of crystal structure at 191°) from acetone, $[\alpha]_D^{25} = +114°$ (*c* = 0.06%, EtOH). *Cudlin et al.* [6] record m.p. 209–210°. – UV. (EtOH): 269 (10560). – ORD. (*c* = 0.055 and 0.61, EtOH): 220 (– 4310), 246 (– 9480), 263 (0), 281 (+ 15515), 308 (0), 316 (– 940), 326 (0), 347 (+ 1815), 400 (+ 1055), 589 (+ 270). – CD. (*c* = 0.011 and 0.055, EtOH): 226 (0), 265 (+ 5.5), 286 (0), 297 (– 2.0), 327 (0), 330–340 (+ 0.4), 360 (0). – IR. (Nujol): 3170 (NH), 3080 (C=CH), 1700 and 1685 (glutarimide CO), 1655 and 1610 (CO and C=C of –OCH=CMeCO). – NMR. ($CDCl_3$): 8.0 (*s*, N–H); 7.20 (br. *s*, H–C(2)); 4.42 (*m*, H–C(6)); 2.80 (*m*, 2H–C(5)); 1.70 (*d*, $J = 1.5$, H_3C–C(3)). – MS. (*m/e*): 237.1000 (M^+, calc. for $C_{12}H_{15}NO_4$ 237.1001), 219 ($M^+ - H_2O$), 209 ($M^+ - CO$), 180 ($M^+ - C_3H_5O$), 154.0866 (calc. for $C_8H_{12}NO_2$ 154.0868), 111 ($C_6H_7O_2$), 85 ($C_4H_5O_2$).

$C_{12}H_{15}NO_4$ (237.25) Calc. C 60.75 H 6.37 N 5.90% Found C 60.8 H 6.55 N 5.69%

Similar treatment of 9-methylstreptimidone yielded the 3-methyl-5,6-dihydro-4-pyrone **8**, identical in every respect with that obtained from streptimidone.

The hemiacetal **7** (R=H) *and the 4-hydroxy-3-methyl-5,6-dihydro-2-pyrone* **9**. Streptimidone (432 mg) in methanol (10 ml) was ozonized to completion at – 78°. After evaporation of the solvent, the residue was dissolved in acetone (9 ml) and oxidized with chromium trioxide (0.8 N in 9 N sulfuric acid, 2.3 ml) at room temp. The unused oxidant was destroyed with methanol, and the solution diluted with water, adjusted to pH 2, and saturated with sodium chloride. After removal of the acetone in vacuum, the aqueous solution was extracted with ethyl acetate. The

extracts were dried, evaporated, and chromatographed on silica gel. Chloroform/methanol eluted first the *hemiacetal* **7** (R=H) (70 mg), colourless plates from acetone/benzene, m.p. 117–128° (with change of crystal structure at 35–70°). – UV. (EtOH): 285 nm, changing in acid to 258 nm and in alkali to 294 nm with greatly increased intensity (ring-opened forms HOCH=CMeCO and ⁻OCH=CMeCO, respectively). – IR. (Nujol): 3430 and 3220 (NH, OH), 1735, 1700 and 1655 (glutarimide CO and ketone CO). – NMR. (CD_3COCD_3): 9.36 (*s*, exchanged with D_2O, N—H); 5.56 (*m*, exchanged with D_2O, H-bonded O—H); 5.44 (*m*, converted to *d*, J = 4 upon addition of D_2O, H—C(2)); 4.40 (*m*, width 30 Hz, H—C(6)); 3.70 (*s*, exchanged with D_2O, free O—H); 0.97 (*d*, J = 6, H_3C—C(3)). – Spin decoupling (D_2O/CD_3COCD_3): irradiation at 2.59 (H—C(3)) converted the *d* at 5.44 (H—C(2)) to a *s*, and the *d* at 0.97 (H_3C—C(3)) to a *s*; irradiation at either 2.31 (2H—C(5)) or 1.69 (2H—C(1′)) converted the *m* at 4.40 (H—C(6)) to a *d* × *d*. – MS. (*m/e*): 255 (M^+, $C_{12}H_{17}NO_5$), 237 ($M^+ - H_2O$), 209.1050 ($M^+ - CH_2O_2$, *m** at 171.3, calc. for $C_{11}H_{15}NO_3$ 209.1052), 199.0844 ($M^+ - C_3H_4O$, calc. for $C_9H_{13}NO_4$ 199.0845), 191.0945 ($M^+ - CH_2O_2 - H_2O$, calc. for $C_{11}H_{13}NO_2$ 191.0946), 180.0663 (calc. for $C_9H_{10}NO_3$ 180.0661), 153.0786 (calc. for $C_8H_{11}NO_2$ 153.0790).

$C_{12}H_{17}NO_5$ (255.26) Calc. C 56.46 H 6.71 N 5.49% Found C 56.5 H 6.84 N 5.24%

Further elution with the same solvent gave the *4-hydroxy-3-methyl-5,6-dihydro-2-pyrone* **9** (80 mg), m.p. 145–147° from methanol, $[\alpha]_D^{25} = -790°$ (*c* = 0.0059%, EtOH). – UV. (EtOH): 248 (13750) in acidic solution, 280 (25570) upon addition of alkali. – ORD. (*c* = 0.059, EtOH): 220 (+20025), 236 (+31650), 250 (0), 262 (−18790), 300 (−3230), 589 (−1990). – CD. (*c* = 0.024, EtOH): 218 (+1.6), 225 (0), 250 (−11.7), 275 (0). – IR. (Nujol): 3330 and 3170 (NH, OH), 1730, 1690 and 1640 (glutarimide CO and —HOC=$CMeCO_2$—). – NMR. (CD_3OD): 4.52 (*m*, H—C(6)); 1.72 (*s*, H_3C—C(3)). – MS. (*m/e*): 253 (M^+, $C_{12}H_{15}NO_5$), 209 ($M^+ - CO_2$), 180 ($C_9H_{10}NO_3$).

$C_{12}H_{15}NO_5$ (253.25) Calc. C 56.91 H 5.97 N 5.53% Found C 56.9 H 5.84 N 5.31%

Similar oxidation of 9-methylstreptimidone afforded the 4-hydroxy-3-methyl-5,6-dihydro-2-pyrone **9**, identical in all respects with that from streptimidone.

Methylation of the 4-hydroxy-3-methyl-5,6-dihydro-2-pyrone **9**. The 5,6-dihydro-2-pyrone **9** (100 mg) in methanol (5 ml) was methylated with ethereal diazomethane, and the residue after evaporation of the solvents was chromatographed on silica gel. Chloroform/methanol eluted first *N,O*-dimethylated isomers. – MS. (*m/e*): 281 (M^+, $C_{14}H_{19}NO_5$).

Further elution yielded the *4-methoxy-3-methyl-5,6-dihydro-2-pyrone* **10** (16 mg), m.p. 208–210° from methanol, $[\alpha]_D^{25} = -40°$ (*c* = 0.043%, EtOH). – UV. (EtOH): 252 (13520). – ORD. (*c* = 0.017 and 0.43, EtOH): 240 (+27270), 257 (0), 269 (−10990), 400 (−390), 589 (−115). – CD. (*c* = 0.0086, EtOH): 220 (positive), 223 (0), 253 (−9.2), 280 (0). – IR. (Nujol): 3180 (NH), 1735, 1695, 1675 and 1640 (glutarimide CO and —MeOC=$CMeCO_2$—). – NMR. ($CDCl_3/CD_3OD$): 4.47 (*m*, width 30 Hz, H—C(6)); 3.83 (*s*, CH_3—O); 1.76 (*d*, J = 0.7, H_3C—C(3)). – MS. (*m/e*): 267.1106 (M^+, calc. for $C_{13}H_{17}NO_5$ 267.1106), 249.0997 ($M^+ - H_2O$, calc. for $C_{13}H_{15}NO_4$ 249.1001), 234 ($M^+ - H_2O - CH_3$), 223 ($M^+ - CO_2$), 191 ($M^+ - CO_2 - CH_3OH$), 180 ($C_9H_{10}NO_3$), 141.0551 (calc. for $C_7H_9O_3$ 141.0551).

$C_{13}H_{17}NO_5$ (267.27) Calc. C 58.42 H 6.41 N 5.24% Found C 58.7 H 6.41 N 5.13%

The same solvent then eluted the *2-methoxy-3-methyl-5,6-dihydro-4-pyrone* **11** (16 mg), m.p. 229–231° from methanol, $[\alpha]_D^{25}$ + 75° (*c* = 0.038%, EtOH). – UV. (EtOH): 276 (15515). – ORD. (*c* = 0.015 and 0.38, EtOH): 225 (+18735), 250 (+23885), 265 (0), 280 (−51985), 293 (0), 303 (+22010), 400 (+1120), 589 (+205). – CD. (*c* = 0.015, EtOH): 232 (0), 268 (−13.1), 281 (0), 292 (+13.6), 323 (0). – IR. (Nujol): 3130 (NH), 1720, 1700 and 1690 (glutarimide CO and —O(MeO)C=CMeCO). – NMR. ($CDCl_3/CD_3OD$): 4.64 (*m*, width 30 Hz, H—C(6)); 3.90 (*s*, CH_3—O); 1.65 (*s*, H_3C—C(3)). – Spin decoupling: irradiation at 2.43 (2H—C(5)) converted the *m* at 4.64 (H—C(6)) to a *d* × *d*. – MS. (*m/e*): 267.1103 (M^+, calc. for $C_{13}H_{17}NO_5$ 267.1106), 207.0892 ($M^+ - C_2H_4O_2$, calc. for $C_{11}H_{13}NO_3$ 207.0895), 180 ($C_9H_{10}NO_3$), 141.0543 (calc. for $C_7H_9O_3$ 141.0552), 114.0313 (calc. for $C_5H_6O_3$ 114.0316).

$C_{13}H_{17}NO_5$ (267.27) Calc. C 58.42 H 6.41 N 5.24% Found C 58.0 H 6.45 N 4.96%

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