950. *Carbohydrate Components of Antibiotics. Part I. Degradation of Desosamine by Alkali: its Absolute Configuration at Position 5.*

By C. H. Bolton, A. B. Foster, M. Stacey, and J. M. Webber.

Alkaline degradation of desosamine, a 3,4,6-trideoxy-3-dimethylaminohexose, yields (*erythro* + *threo*)-2,5-dihydroxyhexanoic acid. This acid is further degraded to (−)-pentane-1,4-diol containing the asymmetric carbon atom which was originally $C_{(5)}$ of desosamine. The absolute configuration of (−)-pentane-1,4-diol is correlated with that of D-glyceraldehyde, thereby indicating that desosamine is a D-hexose derivative.

Several 3-deoxy-3-dimethylaminohexose derivatives have now been recognised as structural components of antibiotics. Desosamine (picrocin), a 3,4,6-trideoxy-3-dimethylaminohexose, forms part of the macrolide antibiotics erythromycin,[1] methymycin,[2] narbomycin,[3] oleandomycin,[4] and picromycin.[5] Carbomycin (magnamycin),[6] also a macrolide antibiotic, contains mycaminose, a 3,6-dideoxy-3-dimethylaminohexose. Amosamine, present in amicetin,[7] is probably [8,9] also a 3,6-dideoxy-3-dimethylaminohexose; amosamine and mycaminose exhibit markedly different mobilities in paper chromatography.[9] Rhodomycin [10] contains rhodosamine which is believed to be a 2,3,6-trideoxy-3-dimethylaminohexose. Although a partial relative configuration has been deduced for mycaminose,[11] its absolute configuration, and that of the other 3-deoxy-3-dimethylaminohexose derivatives, have not been established. We now report a partial configurational assignment for desosamine.

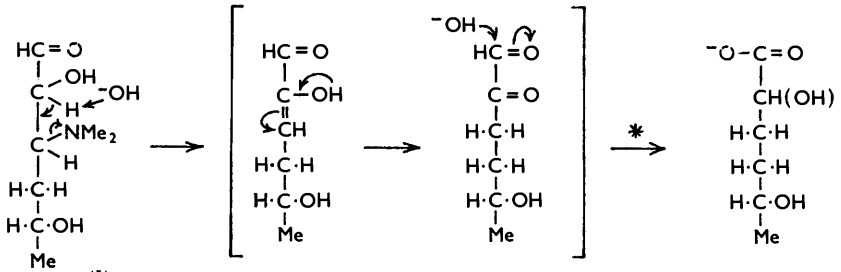
Allocation of the skeletal structure of desosamine (and of the other 3-deoxy-3-dimethylaminohexose derivatives) is based mainly on the pattern of periodate oxidation [12,13] and on the ease of elimination of dimethylamine during treatment with alkali.[13] The other products of the latter reaction, which is indicative [6] of a β-amino-carbonyl structure, have apparently not been identified although a mechanism has been postulated [11] for the alkaline degradation of mycaminose. Identification of the products in the case of desosamine has now permitted both determination of the absolute configuration at position 5 and confirmation of the skeletal structure.

Desosamine hydrochloride was readily isolated in *ca.* 60% yield after vigorous acidic hydrolysis of erythromycin. Dimethylamine (91% in *ca.* 24 hr.) was evolved when the sugar was treated with 0·05N-sodium hydroxide at 50°. When the degradation was performed with oxygen-free lime water at 25°, 6—7 days were required for complete reaction. The same acidic product (isolated as the calcium salt) was formed in each degradation and, by analogy with the products of the alkaline decomposition of 3-*O*-substituted hexoses,[14] it was expected to be calcium (*erythro* + *threo*)-2,5-dihydroxyhexanoate arising by the mechanism [11] (I) ⟶ (II). An alternative mechanism analogous

[1] Clark, *Antibiotics and Chemotherapy*, 1953, **3**, 663; Wiley, Gerzon, Flynn, Sigal, Weaver, Quarck Chauvette, and Monahan, *J. Amer. Chem. Soc.*, 1957, **79**, 6062.
[2] Djerassi and Zderic, *J. Amer. Chem. Soc.*, 1956, **78**, 6390; Djerassi and Halpern, *ibid.*, 1957, **79**, 2022.
[3] Corbaz, Ettlinger, Gäumann, Keller, Kradolfer, Kyburz, Neipp, Prelog, Reusser, and Zähner, *Helv. Chim. Acta*, 1955, **38**, 935.
[4] Els, Celmer, and Murai, *J. Amer. Chem. Soc.*, 1958, **80**, 3777.
[5] Brockmann and Oster, *Chem. Ber.*, 1957, **90**, 605; Anliker and Gubler, *Helv. Chim. Acta*, 1957, **40**, 119.
[6] Hochstein and Regna, *J. Amer. Chem. Soc.*, 1955, **77**, 3353.
[7] Stevens, Gasser, Mukherjee, and Haskell, *J. Amer. Chem. Soc.*, 1956, **78**, 6212.
[8] Professor C. L. Stevens, personal communication.
[9] Foster, Lehmann, Webber, and Westwood, unpublished results.
[10] Brockmann and Borchers, *Chem. Ber.*, 1953, **86**, 261; Brockmann and Spohler, *Naturwiss.*, 1955, **42**, 154.
[11] Woodward, *Angew. Chem.*, 1957, **69**, 50.
[12] Flynn, Sigal, Wiley, and Gerzon, *J. Amer. Chem. Soc.*, 1954, **76**, 3121.
[13] Brockmann, König, and Oster, *Chem. Ber.*, 1954, **87**, 856.
[14] Whistler and BeMiller, *Adv. Carbohydrate Chem.*, 1958, **13**, 289.

to that given [15] for the alkaline degradation of 3-*O*-methyl-D-glucose may also be invoked.

The structure of the calcium salt was proved as follows. Removal of the cations, and reduction of the resultant lactone with lithium aluminium hydride, afforded a (—)-hexanetriol which rapidly consumed 1 mol. of periodate with release of 1 mol. of formaldehyde, and



(I) (II)

* Benzilic acid rearrangement.

therefore contained the ·CH(OH)·CH_2·OH grouping with the third hydroxyl group not vicinal. The (—)-hexanetriol had mobilities in paper chromatography, and in ionophoresis in a borate buffer at pH 10, similar to those of hexane-1,2,6-triol, but the two triols were chemically different. That the (—)-hexanetriol contained an unbranched carbon chain, and a 1,2,5-, not a 1,2,4-, distribution of the hydroxyl groups, followed from the isolation of (—)-pentane-1,4-diol on reduction, with borohydride, of the products of periodate oxidation. Two lines of evidence indicated a 1,4-distribution of the hydroxyl groups in the optically active diol. First, the diol did not form a complex with borate ions as shown by the zero M_G value in paper ionophoresis in alkaline borate, behaviour characteristic of 1,4-diols whereas hexane-1,2,4-triol would have been degraded to pentane-1,3-diol and Frahn and Mills [16] have found several acyclic 1,3-diols to have M_G values in the range 0·05—0·24. Secondly, a *ca.* 0·004M-solution of the (—)-pentanediol in carbon tetrachloride had $\nu_{max.}$ at 3634 (ε 33) and 3479 cm.$^{-1}$ (ε 15) for free and intramolecularly bonded hydroxyl groups,[17] respectively, an absorption pattern closely similar to that of butane-1,4-diol [18] [$\nu_{max.}$ 3640 (ε 29) and 3484 cm.$^{-1}$ (ε 11)]. The Δν values [17] for these diols (155 and 156 respectively) are characteristic of intramolecular hydrogen bonds in 1,4-diols and quite different from those [18] for comparable 1,2- and 1,3-diols (*e.g.*, ethane-1,2-diol Δν 26, and propane-1,3-diol Δν 76). Since the (—)-pentane-1,4-diol and authentic racemic pentane-1,4-diol gave indistinguishable infrared spectra (liquid films) the identity of the former compound was established. Racemic pentane-1,4-diol was readily obtained by the reduction of lævulic acid with lithium aluminium hydride.

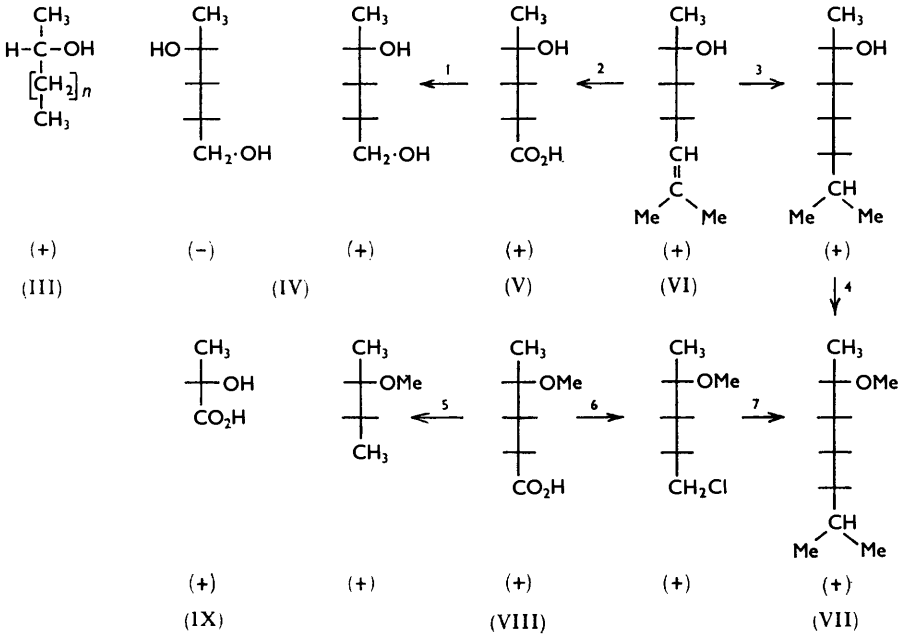
The (—)-hexane-1,2,5-triol gave a tri-(*p*-phenylazobenzoate) with a diffuse melting point which was not sharpened by repeated recrystallisation. This is strong evidence for the presence of a mixture of *erythro*- and *threo*-isomers, since *p*-phenylazobenzoates of pure and racemic alcohols generally exhibit well-defined melting points.[19] Further relevant examples are (—)-pentane-1,4-diol and the racemic alcohols, hexane-1,2,6-triol and pentane-1,4-diol, all of which gave sharp melting *p*-phenylazobenzoates. It follows that the immediate precursor of the (—)-hexane-1,2,5-triol was (*erythro* + *threo*)-2,5-dihydroxyhexanoic acid. No evidence was obtained which indicated the proportions of isomers in this product. The formation of isomers is more easily demonstrated in the alkaline degradation of mycaminose. Reduction with sodium borohydride [20] of the lactone isolated

[15] Kenner and Richards, *J.*, 1954, 278.
[16] Frahn and Mills, *Austral. J. Chem.*, 1959, **12**, 65.
[17] Kuhn, *J. Amer. Chem. Soc.*, 1952, **74**, 2492; 1954, **76**, 4323.
[18] Foster, Haines, and Stacey, *Tetrahedron*, in the press.
[19] Baggett, Foster, Haines, and Stacey, *J.*, 1960, 3528.
[20] Wolfrom and Wood, *J. Amer. Chem. Soc.*, 1951, **73**, 2933; Wolfrom and Anno, *ibid.*, 1952, **74**, 5583.

after the alkaline degradation gave a mixture of 3,6-dideoxy-*arabino*- and -*ribo*-hexose, easily separable by paper ionophoresis in an alkaline borate buffer.[21] It is of interest that the proportion of isomers in the mixture of α- and β-D-glucometasaccharinic acids formed by the alkaline degradation of 3-*O*-benzyl-D-glucose varies according to the experimental conditions.[22] Establishment of the structures of the sequence of products obtained after the alkaline degradation of desosamine also substantiates the structure allocated to the amino-sugar derivative.

Although the yield of dimethylamine from the degradation of desosamine with sodium hydroxide was high (91%), the yield of acidic products was much lower (65%). The difference was accounted for by a second, unidentified, non-acidic product, that was readily separated by chromatography on a cellulose column.

The (—)-pentane-1,4-diol described above contains one asymmetric carbon atom which remained unaffected in the sequence of reactions by which the diol was derived from desosamine. The absolute configuration of (—)-pentane-1,4-diol, which showed a plain negative rotatory dispersion, can be deduced in two ways. First, it is known[23] that compounds having the absolute configuration (III) are dextrorotatory. It is reasonable to suppose that since the hydroxymethyl group in (—)-pentane-1,4-diol is relatively remote



1,* Reduction of acid lactone with sodium and acetic acid.[25] 2, Ozonolysis, followed by oxidation of the resulting aldehyde with silver oxide.[28] 3, Catalytic hydrogenation.[28] 4,* Methylation of potassium alkoxide with methyl iodide.[26] 5,* Bromination decarboxylation of the silver salt, followed by decomposition of the Grignard salt of the product with water.[26] 6, Reduction with lithium aluminium hydride to give (+)-4-methoxypentan-1-ol, followed by treatment with thionyl chloride.[26] 7, Condensation of the Grignard salt with acetone, followed by dehydration, to give (+)-6-methoxy-2-methylhept-2-ene, and catalytic hydrogenation.[26]

For clarity, all stages in this scheme are depicted with (+)-enantiomorphs; stages marked * were actually performed with (—)-enantiomorphs.

from the asymmetric carbon atom it will have little effect on the optical activity. This being so, the (—)-pentane-1,4-diol can be assigned configuration (IV). Secondly, by

[21] Foster, Lehmann, and Stacey, unpublished results.
[22] Machell and Richards, *J.*, 1960, 1938.
[23] Mills and Klyne, " Progress in Stereochemistry," Butterworths Scientific Publ., London, 1954, Vol. I, p. 177; Klyne, " Determination of Organic Structure by Physical Methods," Academic Press Inc., New York, 1955, p. 73.

reference to the literature, a correlation between the configurations of (—)-pentane-1,4-diol and (—)-lactic acid may be established. Levene and Haller [24] resolved 4-hydroxypentanoic acid (V), prepared by reduction of lævulic acid, and correlated the configuration of the dextrorotatory acid with that of (+)-lactic acid (IX). Since further reduction of (—)-(V) gave (—)-pentane-1,4-diol (IV),[25] the latter was assigned the same configuration as (—)-lactic acid. Doering and Young [26] have, however, suggested that several of the stages in Levene's correlations are unreliable because of possible participation by the asymmetric centre in reactions involving a neighbouring group. Consequently, in their investigations, methyl ethers of the secondary alcohol intermediates were employed so that any involvement of the asymmetric centre could be more easily detected. In this way, the configuration of (—)-4-methoxypentanoic acid [(—)-(VIII), obtained by partial resolution of the racemic acid] was correlated with that of (—)-lactic acid.[26,27] Although the direct conversion of (—)-(V) into (—)-(VIII) has not been reported in the literature, a configurational relationship can be established [26,28] through the sequence of correlations (VIII) ⟶ (VII), (VI) ⟶ (VII), and (VI) ⟶ (V), in each stage of which the configuration at the asymmetric centre remains unaffected. It follows from the configurational correlation of (—)-lactic acid and D(+)-glyceraldehyde [29] that the asymmetric carbon atom in (—)-pentane-1,4-diol is also related to D-glyceraldehyde. Since this asymmetric carbon atom was originally $C_{(5)}$ in desosamine, the amino-sugar may be allocated to the D-series.

Experimental

Paper electrophoresis was performed on Whatman No. 3 paper by the enclosed strip technique [30] with a borate buffer (pH 10); [31] detection was effected with ammoniacal silver nitrate (prepared from 10% silver nitate solution by addition of a small amount of 2N-sodium hydroxide and sufficient concentrated ammonia just to dissolve the resultant precipitate). Paper chromatography of acids was performed on Whatman No. 1 paper by downward irrigation with the organic phase of (1) ethyl acetate–acetic acid–water (3 : 1 : 1) or (2) isopentyl alcohol–5M-formic acid (1 : 1). For detection, chromatograms were freed from acid by air-drying and sprayed with Chlorophenol Red.[32]

Isolation of Desosamine Hydrochloride.—In a modification of the method of Flynn and his co-workers,[12] a solution of erythromycin (40 g.) in ethanol (240 ml.) was boiled under reflux with 6N-hydrochloric acid (640 ml.) for 4 hr. The solution was cooled, decanted from a tar, continuously extracted with chloroform overnight, decolorised with charcoal, and concentrated. Crystallisation of the syrupy residue from ethanol–ether (at −10°) gave desosamine hydrochloride (6·4 g.), which after recrystallisation was obtained as colourless needles, m. p. 189—190° (decomp.), $[\alpha]_D^{20}$ +50·7° (equilibrium) (*c* 2·03 in H_2O). Flynn *et al.*[12] record m. p. 183—184°.

Degradation of Desosamine with Sodium Hydroxide.—(*a*) A solution of desosamine hydrochloride (0·21 g.) in water (5 ml.) and N-sodium hydroxide (20 ml.) was stored at room temperature while evolved gases were swept into 0·05N-hydrochloric acid (100 ml.) by a current of nitrogen gas. At intervals, aliquot parts (5 ml.) of the acid solution were titrated against 0·05N-sodium hydroxide. After 3 hr., when only 15% of the theoretical quantity of dimethylamine had been evolved, the reaction temperature was raised to 50°; after a further 20 hr., 91% of the dimethylamine had been released and the reaction was terminated. The decreased alkalinity of the reaction mixture (titration of a 1 ml. aliquot part with standard acid) indicated a 65% conversion of desosamine into acidic products. The cooled solution was passed through a column of Amberlite IR-120 resin (H^+ form; 100 ml.), thereafter treated with a chloroform

[24] Levene and Haller, *J. Biol. Chem.*, 1925, **65**, 49; 1926, **67**, 329; 1926, **69**, 165, 569.
[25] Levene, Haller, and Walti, *J. Biol. Chem.*, 1927, **72**, 591.
[26] Doering and Young, *J. Amer. Chem. Soc.*, 1952, **74**, 2997.
[27] Wiberg, *J. Amer. Chem. Soc.*, 1952, **74**, 3891.
[28] Levene and Haller, *J. Biol. Chem.*, 1929, **83**, 177.
[29] Wolfrom, Lemieux, Olin, and Weisblat, *J. Amer. Chem. Soc.*, 1949, **71**, 4057.
[30] Foster, *Chem. and Ind.*, 1952, 1050.
[31] Foster, Newton-Hearn, and Stacey, *J.*, 1956, 30.
[32] Brown, *Nature*, 1951, **167**, 441.

solution of methyldi-n-octylamine (5% v/v) to remove mineral acid,[33] heated under reflux for 18 hr. with an excess of calcium carbonate, filtered, and then dried in the frozen state. The infrared spectra (KCl discs) of this product and that obtained by degradation of desosamine with lime-water were indistinguishable, and showed $\nu_{max.}$ at 1600 (CO_2^-) and 3600 cm.$^{-1}$ (free OH).

(*b*) In a similar degradation, desosamine hydrochloride (3·5 g.) was treated with N-sodium hydroxide (350 ml.) at 50° for 48 hr. After cations and hydrochloric acid had been removed, the solution was concentrated to give a colourless syrup (2·28 g.) which was examined by paper chromatography. In addition to an acidic product [compound *A*, R_F 0·75 and 0·60 in solvents (1) and (2), respectively], a second component (compound *B*) was detected with Chlorophenol Red as a bright blue spot at the solvent front with both systems. A similar mixture of degradation products (3·47 g.) was separated on a cellulose column (55 × 4·5 cm.) by using solvent (2). Chromatographically homogeneous compound *B* (1·135 g.) was eluted first, and was followed by a mixture of compounds *A* and *B*, and then homogeneous compound *A* (0·945 g.). Refractionation of the mixed products yielded additional pure compound *A* (0·23 g.). Compound *A* showed $\nu_{max.}$ (liquid film) at 1740 (δ-lactone C=O) and 3600 cm.$^{-1}$ (free OH), and was tentatively identified as the δ-lactone of 2,5-dihydroxyhexanoic acid. Compound *B* showed $\nu_{max.}$ at, *inter alia*, 1600, 1660, 1705, and 3300—3500 cm.$^{-1}$; it was not examined further.

(−)-*Hexane*-1,2,5-*triol*.—A solution of compound *A* (0·84 g.) in tetrahydrofuran (50 ml.) was added dropwise to a stirred suspension of lithium aluminium hydride (1 g.) in tetrahydrofuran (250 ml.). The mixture was heated under reflux for 4 hr., and the excess of reductant in the cooled solution was then decomposed with water. The filtered solution was concentrated (200 ml.), deionised with Amberlite IR-120 (H^+ form; 500 ml.) and Deacidite E (CO_3^{2-} form; 30 ml.), and again concentrated. Distillation of the crude product (0·55 g.), $[\alpha]_D$ −6·8° (*c* 18·3 in MeOH) gave (−)-*hexane*-1,2,5-*triol* (0·118 g.), b. p. 130—140°/0·5 mm., n_D^{20} 1·4715, $[\alpha]_D^{20}$ −10·2° (*c* 1·18 in H_2O), $[\phi]_D$ −14° (Found: C, 53·4; H, 10·5. $C_6H_{14}O_3$ requires C, 53·7; H, 10·45%), $\nu_{max.}$ (liquid film) 3600 cm.$^{-1}$ (free OH). Paper-electrophoretic examination of this material showed a single spot having the same mobility (M_G 0·20) as hexane-1,2,6-triol, but, in view of its origin, the product probably consisted of a pair of triols, epimeric at $C_{(2)}$. The product gave [19,34] a crystalline *tri*-(p-*phenylazobenzoate*), m. p. 159—169° [from benzene–light petroleum (b. p. 60—80°)] (Found: C, 71·4; H, 4·75; N, 10·9. $C_{45}H_{38}N_6O_6$ requires C, 71·2; H, 5·0; N, 11·1%). Repeated recrystallisation did not raise the m. p.; the infrared spectrum (Nujol mull) showed the absence of free OH. Hexane-1,2,6-triol gave a *tri*-(p-*phenylazobenzoate*), m. p. 144—146° (Found: C, 71·45; H, 5·2; N, 11·1. $C_{45}H_{38}N_6O_6$ requires C, 71·2; H, 5·0; N, 11·1%).

Periodate Oxidation of (−)-*Hexane*-1,2,5-*triol*.—(*a*) A solution of (−)-hexane-1,2,5-triol (0·0185 g.) in water (5 ml.) was treated with 0·25M-sodium metaperiodate (5 ml.), and the volume was rapidly adjusted to 25 ml. The consumption of oxidant was followed by the standard method,[35] and the liberated formaldehyde was determined by the chromotropic acid method.[36] The triol rapidly (6 min.) consumed 1·0 mol. of periodate with release of 1·1 mol. of formaldehyde. Under similar conditions, hexane-1,2,6-triol consumed 0·98 mol. of oxidant and released 1·0 mol. of formaldehyde.

(*b*) After storage for 1 hr., a solution of the 1,2,5-triol (0·20 g.) and sodium metaperiodate (0·35 g., 1·1 mol.) in water (25 ml.) was treated with an aqueous solution (10 ml.) containing barium chloride dihydrate (0·40 g.). Precipitated salts were removed and sodium borohydride (0·10 g.) was added to the solution which was then stored overnight. After the excess of reductant had been destroyed by addition (to pH 6) of 10% acetic acid, the solution was basified (pH 9) with sodium hydrogen carbonate and extracted with ether continuously for 2 days. The residue obtained by concentration (at atmospheric pressure) of the ether extract was dissolved in methanol and the solvent was removed at atmospheric pressure. The dissolution and evaporation were repeated. On distillation, the crude *product*, $[\alpha]_D^{20}$ −13·4° (*c* 1·05 in MeOH) gave D-(−)-pentane-1,4-diol (0·05 g.), b. p. 150—160° (bath)/12 mm. (Found: C, 57·4; H, 11·5. $C_5H_{12}O_2$ requires C, 57·7; H, 11·5%). A solution of the diol (4 mg.) in dry CCl_4 (10 ml.) had $\nu_{max.}$ at 3634 (ε 33) and 3479 cm.$^{-1}$ (ε 15) for free and intramolecularly bonded

[33] Smith and Page, *J. Soc. Chem. Ind.*, 1948, **67**, 48.
[34] Woolfolk, Beach, and McPherson, *J. Org. Chem.*, 1955, **20**, 391.
[35] Jackson, *Org. Reactions*, 1944, **2**, 341.
[36] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.

hydroxyl groups, respectively. The intrument and method used for the measurement of this spectrum are described elsewhere.[37] The diol gave a single spot of zero mobility during paper electrophoresis in borate buffer, and readily formed a *di*-(p-*phenylazobenzoate*), m. p. 118—119·5° [from benzene–light petroleum (b. p. 60—80°)] (Found: C, 71·4; H, 5·3; N, 10·9. $C_{31}H_{28}N_4O_4$ requires C, 71·5; H, 5·4; N, 10·8%). The rotatory dispersion pattern for the diol is recorded below:

λ (mμ)	600	500	400	350	330	320	310	300	290
[φ]	−8°	−12°	−31°	−45°	−52°	−56°	−59°	−67°	−79°

(±)-*Pentane*-1,4-*diol*.—A solution of lævulic acid (5 g.) in tetrahydrofuran (20 ml.) was added dropwise to a stirred suspension of lithium aluminium hydride (5 g.) in tetrahydrofuran (100 ml.). The mixture was heated under reflux overnight and the excess of reductant was decomposed with water. After the solution had been concentrated to remove tetrahydrofuran, it was extracted with chloroform continuously for 2 days, and the extract concentrated. Distillation of the crude product (2·5 g., 55%) gave (±)-pentane-1,4-diol, b. p. 140—145° (bath)/12 mm., n_D^{21} 1·4465. The infrared spectrum (liquid film) of this diol was indistinguishable from that of (−)-pentane-1,4-diol, and showed the following absorptions (cm.$^{-1}$): 3300sb, 2900s, 1430mb, 1370m, 1330mb, 1230w, 1180w, 1130m, 1090m, 1055s, 1035s, 1010s, 995msh, 940m, 900w, 875w, 830w. The (±)-diol gave a crystalline *di*-(p-*phenylazobenzoate*), m. p. 161—162° [from benzene–light petroleum (b. p. 60—80°)] (Found: C, 71·7; H, 5·3. $C_{31}H_{28}N_4O_4$ requires C, 71·5; H, 5·4%).

The authors thank Eli Lilly and Company for generous gifts of erythromycin, and Professor W. Klyne for the optical rotatory dispersion measurements.

Department of Chemistry, The University,
Edgbaston, Birmingham, 15. [*Received, May 17th*, 1961.]

[37] Dobinson and Foster, *J.*, 1961, 2338.