1075. *Synthesis of Chromose A.*

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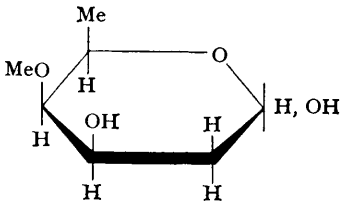
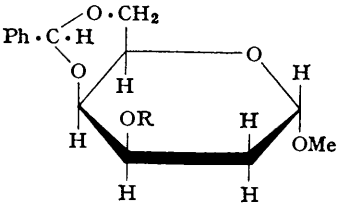
The structure of chromose A, a sugar component of the antitumour substance chromomycin A_3, has been established as 2,6-dideoxy-4-*O*-methyl-D-*lyxo*-hexose by synthesis.

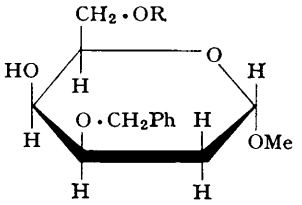
Chromomycin is the name given[1] to a group of cancerostatic and anticancer antibiotics produced by *Streptomyces griseus* No. 7. The principal antibiotic of this group was isolated by Tatsuoka, Miyake, and Mizuno[2] and named chromomycin A_3. Similar antibiotics have been reported[3] by other groups and recent evidence[4] indicates that the principal antibiotic, olivomycin, from *Streptomyces olivoreticuli* resembles chromomycin A_3. Hydrolysis of chromomycin A_3 with 50% acetic acid yielded, *inter alia*, a water-soluble fraction from which four sugars have been isolated;[5] these sugars have been designated as chromose A, B, C, and D, respectively. Physical data reported[4] for olivomose, a sugar component of olivomycin, are similar to those of chromose A. Chromose A has been identified[6] as 2,6-dideoxy-4-*O*-methyl-D-*lyxo*-hexose (2,6-dideoxy-4-*O*-methyl-D-galactose) (I) on the basis of chemical and n.m.r.



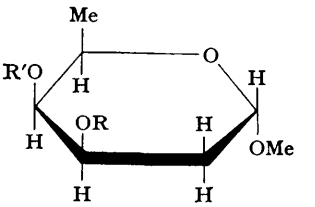
(I)



(II) R = H
(III) R = CH_2Ph



(IV) R = H
(V) R = $SO_2 \cdot C_6H_4Me$



(VI) R = CH_2Ph, R′ = H
(VII) R = CH_2Ph, R′ = Me
(VIII) R = H, R′ = Me

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[1] K. Nakazawa, M. Shibata, K. Tanabe, Y. Tokui, A. Miyake, H. Hitomi, M. Miyamoto, and M. Imanishi, Jap. Pat., 12,646/1960.

[2] S. Tatsuoka, A. Miyake, and K. Mizuno, *J. Antibiotics, Ser. B*, 1960, **13**, 332.

[3] J. E. Phillip and J. R. Schenck, *Antibiotics and Chemotherap.*, 1953, **3**, 1218; R. M. Gale, M. H. Hoehn, and M. H. Cormick, *Antibiotics Ann.*, 1959, 489; K. V. Rao, W. R. Cullen, and B. O. Sobin, *Antibiotics and Chemotherap.*, 1962, **12**, 182; H. Schmitz, B. Heinmann, J. Lein, and I. R. Hooper, *ibid.*, 1960, **10**, 740; P. Sensi, A. M. Greco, and H. Pagani, *ibid.*, 1958, **8**, 241; M. G. Brazhnikova, E. B. Kruglyak, I. N. Kovsharova, N. V. Konstantinova, and V. V. Proshlyakova, *Antibiotiki*, 1962, **7**, 39; H. Nishimura, T. Kimura, K. Tawara, K. Sasaki, K. Nakajima, N. Shimaoka, S. Okamoto, M. Shinohara, and J. Isono, *J. Antibiotics, Ser. A*, 1957, **10**, 205.

[4] Y. A. Berlin, S. E. Esipov, M. N. Kolosov, M. M. Shemyakin, and M. G. Brazhnikova, *Tetrahedron Letters*, 1964, 1323.

[5] S. Tatsuoka, K. Tanaka, M. Miyamoto, K. Morita, Y. Kawamatsu, K. Nakanishi, Y. Nakadaira, and N. S. Bhacca, *Proc. Japan Acad.*, 1964, **40**, 236.

[6] M. Miyamoto, Y. Kawamatsu, M. Shinohara, Y. Asahi, Y. Nakadaira, H. Kakisawa, K. Nakanishi, and N. S. Bhacca, *Tetrahedron Letters*, 1963, 693.

evidence and by application of Hudson's rules and Whiffen's calculations. The following stereospecific synthesis of chromose A confirms this assignment of structure.

Benzylidenation of methyl 2-deoxy-α-D-*lyxo*-hexopyranoside gave the known[7] 4,6-*O*-benzylidene derivative (II), which was converted into methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-*lyxo*-hexopyranoside (III) on treatment with benzyl bromide and sodium hydride in *NN*-dimethylformamide.[8] In view of the lability[9] of 2-deoxy-glycosides to acid, debenzylidenation of compound (III) was accomplished with 0·001N-hydrochloric acid at 80° for 5 hr., to yield methyl 3-*O*-benzyl-2-deoxy-α-D-*lyxo*-hexopyranoside (IV). Monotosylation then afforded syrupy methyl 3-*O*-benzyl-2-deoxy-6-*O*-tosyl-α-D-*lyxo*-hexopyranoside (V), which on desulphonyloxylation with lithium aluminium hydride was converted into methyl 3-*O*-benzyl-2,6-dideoxy-α-D-*lyxo*-hexopyranoside (VI), and thence, by methylation, into methyl 3-*O*-benzyl-2,6-dideoxy-4-*O*-methyl-α-D-*lyxo*-hexopyranoside (VII). The latter compound could also be obtained, although in a chromatographically less pure form, by methylation of compound (V) followed by treatment of the methylated product with lithium aluminium hydride.

Catalytic debenzylation[10] of the glycoside (VII) gave methyl 2,6-dideoxy-4-*O*-methyl-α-D-*lyxo*-hexopyranoside (VIII), m. p. 96—97°, $[\alpha]_D^{25}+174°$ (*c* 1·2 in ethanol). Miyamoto *et al.*[6] report m. p. 92°, $[\alpha]_D^{22}+122°$ (*c* 1 in ethanol) for methyl α-D-chromoside A obtained by methanolysis of chromomycin A_3, and Berlin *et al.*[4] give m. p. 98°, $[\alpha]_D^{26}+150°$ (*c* 0·4 in ethanol) for olivomoside A obtained in a similar manner from olivomycin. The n.m.r. spectrum recorded[6] for methyl α-D-chromoside A contains a quartet at 4·25, which was absent from the spectrum* of the synthetic glycoside, and which can probably be attributed to contamination by the β-anomer {m. p. 152°, $[\alpha]_D^{22}-36°$ (*c* 1 in ethanol)}. This conclusion was supported by a comparison of the infrared spectra of the natural and synthetic glycosides. Apart from slight differences attributable to the presence of the β-anomer, the n.m.r. and infrared spectra of these glycosides were indistinguishable. Hydrolysis of the synthetic glycoside (VIII) with N-hydrochloric acid at room temperature gave 2,6-dideoxy-4-*O*-methyl-D-*lyxo*-hexose (I), m. p. 151—153° (decomp.), $[\alpha]_D^{25}+80°$ (final, *c* 1·1 in water). The melting point of the synthetic sugar was not depressed on admixture with chromose A. Miyamoto *et al.*[6] report m. p. 151°, $[\alpha]_D^{22}+93° \rightarrow +77°$ (final, *c* 1 in water), for chromose A, and Berlin *et al.*[4] give m. p. 158—162°, $[\alpha]_D^{23}+98·5 \rightarrow +89°$ (final, *c* 0·5 in water), for olivomose. The infrared spectrum, *X*-ray powder photograph, and thin-layer chromatographic properties of the synthetic sugar were indistinguishable from those of chromose A.

Experimental

Thin-layer chromatography was performed on silica gel (Merck) using benzene–methanol as the mobile phase. The separated materials were detected by spraying the dried chromatogram with an acidified 3% (w/v) solution of vanillin in ethanol[11] and heating at 115° for 5—10 min. Solvents were usually removed under reduced pressure below 40°.

Methyl 3-O-*Benzyl*-4,6-O-*benzylidene*-2-*deoxy*-α-D-lyxo-*hexopyranoside* (III).—To a solution of methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*lyxo*-hexopyranoside[7] (15 g.) in dry *NN*-dimethylformamide (600 ml.) was added sodium hydride powder (6 g.) in portions, and after 45 min. benzyl bromide (19 ml.) was added dropwise. The mixture was set aside overnight, dry methanol was added to remove unchanged benzyl bromide, and the solvents were removed under reduced pressure. The residue was extracted with chloroform (3 × 100 ml.) and the extracts washed with water and dried ($MgSO_4$). Evaporation of the solvent gave the *product* (15 g.), m. p. 109—110° (from absolute ethanol), $[\alpha]_D^{25}+161°$ (*c* 2 in chloroform) (Found: C, 70·6; H, 6·6. $C_{21}H_{24}O_5$ requires C, 70·75; H, 6·8%).

* Determined in deuterochloroform with tetramethylsilane as internal reference using a Varian A60 spectrometer. Absorptions given on the δ scale.

[7] C. Tamm and T. Reichstein, *Helv. Chim. Acta*, 1948, **31**, 1630.
[8] B. D. Jones and J. J. Willard, personal communication.
[9] W. G. Overend and M. Stacey, *Adv. Carbohydrate Chem.*, 1953, **8**, 45.
[10] R. Mozingo, *Org. Synth.*, 1946, **26**, 77.
[11] E. Merck AG, Darmstadt, "Chromatography," 2nd edn., p. 30.

Methyl 3-O-*Benzyl*-2-*deoxy*-α-D-lyxo-*hexopyranoside* (IV).—A solution of the foregoing compound (5 g.) in aqueous methanol (1:8 v/v; 900 ml.) containing 0·01N-hydrochloric acid (100 ml.) was heated at 80°, and the removal of the benzylidene group followed by thin-layer chromatography. After 5 hr., the cooled solution was neutralised ($PbCO_3$), filtered, and the filtrate evaporated under reduced pressure to a solid residue. Recrystallisation (twice) from ethyl acetate–light petroleum (b. p. 80—100°) gave the *product* (2·3 g.), m. p. 89—90°, $[\alpha]_{Hg}^{20}$ +161° (*c* 1·33 in ethanol) (Found: C, 62·6; H, 7·6. $C_{14}H_{20}O_5$ requires C, 62·7; H, 7·5%).

Methyl 3-O-*Benzyl*-2-*deoxy*-6-O-*tosyl*-α-D-lyxo-*hexopyranoside* (V).—A solution of tosyl chloride (0·78 g.) in dry pyridine (1·7 ml.) was added during 20 min. to a cooled (0°) solution of the foregoing compound (1 g.) in dry pyridine (9 ml.). The solution was allowed to stand at room temperature for 48 hr. and was then evaporated to dryness under reduced pressure. The residue was extracted with chloroform (100 ml.) and this extract was washed with sodium hydrogen sulphite solution (3 × 50 ml.), sodium hydrogen carbonate solution (3 × 50 ml.), and water (3 × 50 ml.), and dried ($MgSO_4$). Removal of the chloroform gave a syrup (1·7 g.) which in addition to the *product* was shown, by thin-layer chromatography, to contain traces of starting material and the ditosylated compound. A chromatographically pure, syrupy *product*, $[\alpha]_D^{25}$ +88° (*c* 1·5 in chloroform), was obtained following chromatography on silica gel (Davidson grade 950, 60—200 mesh) and elution with benzene–ether (1:1 v/v). The infrared spectrum agreed with the structure assigned [$\nu_{max.}$ 3400—3600 (OH), and 1370 and 1190 cm.$^{-1}$ (sulphonate ester)].

Methyl 3-O-*Benzyl*-2-*deoxy*-4,6-*di*-O-*tosyl*-α-D-lyxo-*hexopyranoside*.—A solution of methyl 3-*O*-benzyl-2-deoxy-α-D-*lyxo*-hexopyranoside (50 mg.) and tosyl chloride (150 mg.) in dry pyridine (1 ml.) was allowed to stand for 3 days at room temperature and then for 1 day at 37°. The mixture was worked up as described in the previous experiment and the resultant syrup crystallised on cooling to 0°. Recrystallisation from benzene–light petroleum (b. p. 80—100°) afforded the pure *product* (14 mg.), m. p. 123—125° (decomp.), $[\alpha]_{Hg}^{23}$ +28° (*c* 1·5 in benzene) (Found: C, 58·5; H, 5·7; S, 12·0. $C_{28}H_{32}O_9S_2$ requires C, 58·3; H, 5·6; S, 11·1%).

Methyl 3-O-*Benzyl*-2,6-*dideoxy*-α-D-lyxo-*hexopyranoside* (VI).—A solution of methyl 3-*O*-benzyl-2-deoxy-6-*O*-tosyl-α-D-*lyxo*-hexopyranoside (1·1 g.) in benzene–ether (110 ml., 1:2 v/v) was treated at reflux temperature with lithium aluminium hydride (0·3 g.) for 10 hr. On cooling, ethyl acetate and water were added and insoluble material was filtered off and washed with ether. The combined filtrate and washings were washed with water (2 × 50 ml.) and dried ($MgSO_4$). Removal of the solvents gave the syrupy *product* (0·57 g.), $[\alpha]_D^{20}$ +83° (*c* 2·5 in chloroform), which could not be induced to crystallise. This material was substantially homogeneous, and was readily distinguished from the starting material, on thin-layer chromatograms. Tests for sulphur on the syrup were negative, and absorption bands due to the sulphonate ester grouping were absent from its infrared spectrum.

Methyl 3-O-*Benzyl*-2,6-*dideoxy*-4-O-*methyl*-α-D-lyxo-*hexopyranoside* (VII).—The foregoing compound (0·57 g.) was heated under reflux with freshly distilled methyl iodide (30 ml.) and silver oxide (1·6 g.) for 48 hr. Additional amounts (2 × 1 g.) of silver oxide were added during the reaction. The cooled solution was filtered, the residue washed on the filter with ether, and the combined filtrate and washings were evaporated to a syrup (0·53 g.). Distillation (twice) of the syrup under reduced pressure gave the *product* (0·38 g.), b. p. 190—200° (bath)/0·05 mm., $[\alpha]_D^{20}$ +134° (*c* 1·9 in chloroform) (Found: C, 68·0; H, 8·6. $C_{15}H_{22}O_4$ requires C, 67·6; H, 8·3%).

Methyl 2,6-*Dideoxy*-4-O-*methyl*-α-D-lyxo-*hexopyranoside* (VIII).—A solution of the foregoing compound (0·36 g.) in ethanol (150 ml.) containing palladium–charcoal[10] (0·2 g.) was shaken for 3 days at room temperature with a slight overpressure of hydrogen. The catalyst was filtered off and the filtrate evaporated under reduced pressure to a crystalline residue (0·22 g.), m. p. 90—94°. Recrystallisation (twice) from light petroleum (b. p. 80—100°) gave the *product* (80 mg.), m. p. 96—97°, $[\alpha]_D^{20}$ +174° (*c* 1·2 in ethanol) (Found: C, 54·6; H, 9·0. $C_8H_{16}O_4$ requires C, 54·5; H, 9·15%). A sample of natural methyl α-D-chromoside A on admixture with the synthetic material had m. p. 93—95°. The infrared (KBr discs) and n.m.r. spectra of the two glycosides were indistinguishable except that the spectra of the natural compound contained additional bands due to the presence of traces of the β-anomer. The thin-layer chromatographic properties of the natural and synthetic glycosides were indistinguishable, and no separation of the anomers was achieved in the solvent systems used.

2,6-*Dideoxy*-4-O-*methyl*-D-lyxo-*hexose* (I).—A solution of the foregoing glycoside (60 mg.) was hydrolysed with N-hydrochloric acid (5 ml.) at room temperature for 4 hr. The solution was then neutralised (Ag_2CO_3), filtered, and the filtrate evaporated to a syrup (54 mg.), which crystallised

on cooling. Recrystallisation from ethyl acetate–light petroleum (b. p. 80—100°) gave the *product* (35 mg.), m. p. 151—153° (decomp.) (undepressed on admixture with chromose A), $[\alpha]_D^{25}$ +80° (final, *c* 1·1 in water) (Found: C, 52·1; H, 8·7. $C_7H_{14}O_4$ requires C, 51·8; H, 8·7%). The infrared spectra, *X*-ray powder photographs, and thin-layer chromatographic properties of the synthetic sugar and chromose A were indistinguishable.

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