Metabolites of Proteaceae. Part VIII.[1] The Occurrence of (+)-D-Allose in Nature: Rubropilosin and Pilorubrosin from *Protea rubropilosa* Beard

By **Guido W. Perold,* Peter Beylis,** and **Arthur S. Howard,** Department of Chemistry, University of the Witwatersrand, Johannesburg

The leaves of *Protea rubropilosa* Beard contain (+)-D-allose in the form of the 6-*O*-cinnamate (rubropilosin) and the 6-*O*-benzoate (pilorubrosin) of 2-hydroxy-4-hydroxymethylphenyl β-D-allopyranoside. These constituents are accompanied in the plant by a β-phenylpropionate of the same glycoside.

In the family Proteaceae, the genera *Leucadendron* and *Leucospermum* typically contain *C*-glycosyl compounds of the leucodrin type, together with arbutin as a representative phenyl glycoside.[1] In the genus *Protea* we have shown [1] that *Protea lacticolor* Salisb. contains the 6-*O*-benzoate (lacticolorin) of 2-hydroxy-4-hydroxymethylphenyl β-D-glucopyranoside as a major leaf constituent. It was therefore of considerable interest to find, as announced [2] earlier, that *Protea rubropilosa* Beard, a summer-rainfall *Protea* of the Eastern Transvaal, contains a related set of esters of a 2-hydroxy-4-hydroxymethylphenyl glycoside; the sugar involved, however, is here (+)-D-allose, and this represents the first proven occurrence of this aldohexose, hitherto known only as a synthetic product, in a higher plant. The natural occurrence of other rare aldohexoses (D-talose, D-altrose, and an allose of undetermined configuration) [3] has thus far been demonstrated only in lower order plants such as algae and fungi. The pertinent observation was made [4] in 1965 that 'among the hexoses, the three most stable ones, glucose, mannose, and galactose, are very widely spread. (It is

[1] Part VII, G. W. Perold, P. Beylis, and A. S. Howard, preceding paper.

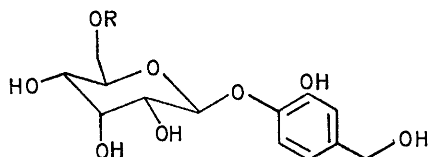
[2] P. Beylis, A. S. Howard, and G. W. Perold, *Chem. Comm.*, 1971, 597.

[3] H. Kauss, *Z. Pflanzenphysiol.*, 1965, **53**, 58; N. Cagnoli Bellavita, P. Ceccherelli, M. Ribaldi, Z. Baskevitch, and J. Polonsky, *Gazzetta*, 1967, **97**, 1344, 1625; N. Cagnoli Bellavita, P. Ceccherelli, M. Ribaldi, J. Polonsky, and Z. Baskevitch, *ibid.*, 1969, **99**, 1354; J. Polonsky, Z. Baskevitch, N. Cagnoli Bellavita, and P. Ceccherelli, *Chem. Comm.*, 1968, **22**, 1404.

[4] E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, 'Conformational Analysis,' Interscience, New York, 1965, p. 432.

curious, however, that allose, of only slightly higher free energy, seems to be unknown in Nature.)'. The present finding thus supports the usefulness of the relative free energy content [5] of such metabolic products as a measure of the probability of their natural occurrence.

The methanolic extract of the leaves of *Protea rubropilosa* Beard contained three kinds of phenol, which appeared on a paper chromatogram (see Experimental



(I) R = PhCH:CH·CO
(II) R = Bz
(III) R = Ph[CH$_2$]$_2$·CO
(IV) R = H

section) as three spots, PR1—3 in increasing order of polarity. PR1 was by far the major component and was shown to consist of rubropilosin (I), pilorubrosin (II), and a third constituent which (see later) is probably the dihydrorubropilosin (III). Rubropilosin and pilorubrosin could be separated efficiently by fractional crystallisation and were obtained from the dried leaves in 0·59 and 0·52% yields, respectively.

Rubropilosin, $C_{22}H_{24}O_9$, m.p. 97—100°, $[\alpha]_D$ −67°, and pilorubrosin, $C_{20}H_{22}O_9$, m.p. 167—169°, $[\alpha]_D$ −66°, are (see Experimental section) hydroxy-esters: the low [6,7] value of the aryl ester stretching vibration near 1685 cm^{-1} (in potassium bromide dispersion) is due to hydrogen bonding (see later); hydrolysis of each of these esters with barium hydroxide solution yielded cinnamic acid [from (I)] and benzoic acid [from (II)]; both compounds furthermore furnished penta-*O*-methyl ethers (see Experimental section) of the expected composition. The same deacylated glycoside, rubropiloside (IV), $C_{13}H_{18}O_8$, m.p. 185—186°, $[\alpha]_D$ −73°, resulted from the alkaline hydrolysis of both (I) and (II), and, by the preparation from it of a hexa-*O*-methyl ether, was shown to contain six hydroxy-groups. As in the case of lacticolorin (from *Protea lacticolor* Salisb.),[1] the aglycone of rubropiloside was too sensitive to allow isolation as such after hydrolysis with hot aqueous acid. It was therefore characterised, in the same way as before,[1] by catalytic hydrogenolysis of the aryl hydroxymethyl group of the glycoside to a methyl group, followed by treatment of the hydrogenolysis product of the glycoside with diazomethane and followed by acidic hydrolysis. The phenolic product obtained was shown to be 4-hydroxy-3-methoxytoluene (see Experimental section), thus confirming both the nature of the aglycone in the native compound as 3,4-dihydroxybenzyl alcohol, and also its point of attachment to the sugar through the hydroxy-group *para* to the hydroxymethyl group.

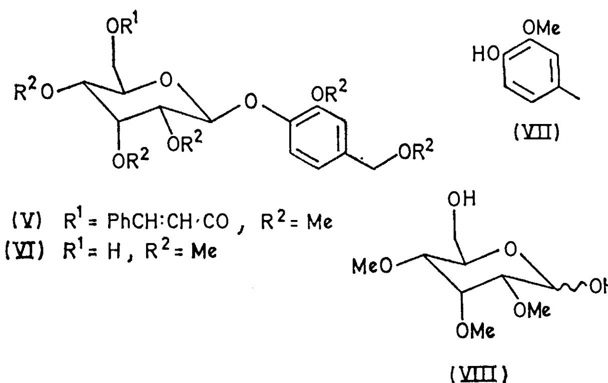
The sugar obtained by acidic hydrolysis of rubropiloside (IV) was crystalline (m.p. 130—132°, $[\alpha]_D$ +14·4°) and was identified as (+)-D-allose by mixed m.p., i.r. spectra, g.l.c. analysis of its trimethylsilyl ether, and *X*-ray powder diffraction data (Table 1); the *p*-bromophenylhydrazone [8] and the osazone prepared from it were furthermore each identical with the corresponding derivatives prepared from authentic β-D-allose and from a sample of β-D-allose, here prepared [9] from (−)-D-ribose.

TABLE 1

Main interplanar spacings (Cu-K_α radiation) in Å

Natural allose	Synthesized β-D-allose	Lit.[10]
7·19	7·21	7·18
5·45	5·40	5·43
4·60	4·60	4·60
4·15	4·18	4·21
3·96	3·99	4·02
3·59	3·59	3·61
3·12	3·13	3·14
2·88	2·88	2·89
2·71	2·71	2·72
2·38	2·38	2·40
2·29	2·30	
1·98	1·99	2·00

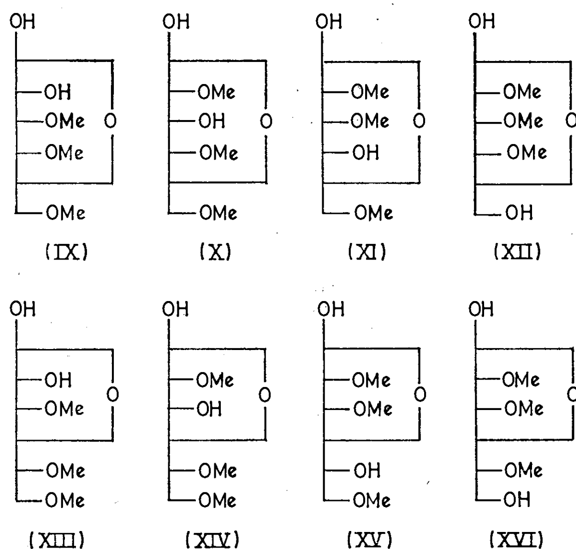
The full structure of rubropilosin was elucidated by degradation of its penta-*O*-methyl ether (V). Hydrolysis with hot barium hydroxide solution gave cinnamic acid and a penta-*O*-methylrubropiloside (VI). Hydrogenolysis of (VI) followed by acidic hydrolysis yielded 4-hydroxy-3-methoxytoluene (VII) and a tri-*O*-methyl-D-allose, m.p. 106—108°, $[\alpha]_D$ +25°.



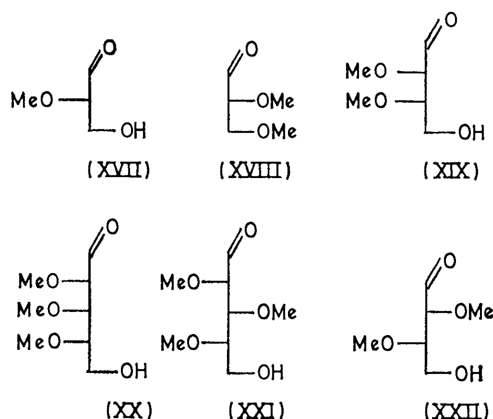
No reference materials were available for identifying the latter compound by direct comparison; it was therefore identified by applying the method of Lemieux and Bauer,[11] using the two tests of oxidation with periodate, and of reduction with sodium borohydride followed by oxidation with periodate (for details see Experimental

[5] Ref. 4, p. 403.
[6] L. J. Bellamy, 'The Infrared Spectra of Complex Molecules,' 2nd edn., Methuen, London, 1962, p. 179.
[7] K. Nakanishi, 'Infrared Absorption Spectroscopy—Practical,' Holden-Day, San Francisco, 1966, p. 44.
[8] F. L. Humoller, *Methods Carbohydrate Chem.*, 1962, **1**, 104.
[9] Ref. 8, p. 102.
[10] M. L. Wolfrom, J. N. Schumacher, H. S. Isbell, and F. L. Humoller, *J. Amer. Chem. Soc.*, 1954, **76**, 5816.
[11] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

section); products were, however, analysed by thin-layer instead of by paper [11] chromatography. The tri-*O*-methyl-D-allose could be one of eight isomers, *viz.* one of four allopyranoside (IX)—(XII) or four allofuranoside structures (XIII)—(XVI). It was not as such oxidizable by periodate and therefore was neither (IX) nor (XIII); after reduction to the corresponding tri-*O*-methylallitol, the product was oxidized by periodate [and hence was derived neither from (X) nor from (XVI)] to yield a product identified by thin-layer behaviour and reagent colouration. This product could now be a mixture of



trioses [(XVII) and (XVIII)] from (XIV), a tetraose (XIX) from either (XI) or (XV), or a pentaose (XX)



from (XII); it was shown to be the last by comparison with similar products [the pentaose (XXI) and the tetraose (XXII)] derived in the same way from 2,3,4- and 2,3,6-tri-*O*-methyl-D-glucose (see Experimental section).

If acyl group migration or ring alteration during the methylation step may be discounted,[1] the established structure of the 2,3,4-tri-*O*-methylallose (VIII) then shows that the ester group in rubropilosin is in position 6, and that the sugar is in the pyranose form. The appearance in the mass spectrum of rubropilosin of a peak at *m/e* 293 for a cinnamoylallose oxonium ion ($C_{15}H_{17}O_6^+$) confirms (as before) [1] that the ester group is attached to the sugar system. In analogy with the study of lacticolorin, rubropilosin penta-acetate was prepared for mass spectral analysis: the greatly enhanced stability of the cinnamoyl ion, *vis-à-vis* the benzoyl ion, here however leads to more ready α-cleavage of this ester group in further fragmentation, so that diagnosis on this basis is less certain than in the case of the related benzoyl compound, lacticolorin. The penta-acetate of the benzoyl analogue, pilorubrosin, does however show fragmentation behaviour similar to that found for the lacticolorin derivative: for the penta-acetates of lacticolorin, pilorubrosin, and rubropilosin the relevant ions are at *m/e* 231 (28%), 231 (16%), and 257 (2%). In the mass spectrum of the rubropilosin penta-*O*-methyl ether (V) the occurrence of a peak at *m/e* 88 (relative intensity 19%) did, however, as before,[1] indicate that the sugar was here present in the pyranose form. This was confirmed by the occurrence of the same peak (at relative intensity 16%) in the mass spectrum of rubropiloside hexa-*O*-methyl ether [as (V), with $R^1 = R^2 =$ Me].

The structure of pilorubrosin was proved in the same way as for rubropilosin, by hydrogenolysis of its penta-*O*-methyl ether and acidic hydrolysis to yield 4-hydroxy-3-methoxytoluene (VII) and the same 2,3,4-tri-*O*-methyl-D-allopyranose (VIII), all data being in agreement with structure (II).

The configuration at the anomeric centre in the two plant metabolites was shown to be β by the value of the coupling constant between H-1 and the axial H-2 of the allose system, expected to be 7·4 Hz or more,[12,13] for such an axial–axial coupling (see Table 2). The o.r.d.

TABLE 2

Chemical shift (δ in p.p.m. relative to Me_4Si) and coupling constant (*J* in Hz) for the H-1 doublet

Compound	Solvent	δ	*J*
Rubropilosin (I)	C_2D_6SO	4·98	8
Pilorubrosin (II)	C_2D_6SO	5·0	7 *
Pilorubrosin (II)	CD_3OD	5·14	7
Rubropiloside (IV)	C_2D_6SO	4·95	8 *

* The doublet appeared clearly only after adding D_2O.

curves of pilorubrosin and rubropiloside are furthermore similar, both displaying a negative Cotton effect (first extremum for both near 290 nm) on a negative plain curve. The ester substituent thus has little effect on the o.r.d. behaviour of the glycoside, so that a comparison with the phenyl D-allopyranosides with regard to their specific rotation is valid: the known values for $[\alpha]_D$ in these cases (for the β-anomer, −54° in pyridine; for the α-anomer, +179° in water),[14] compared with the values

[12] J. M. van der Veen, *J. Org. Chem.*, 1963, **28**, 564.
[13] N. S. Bhacca and D. H. Williams, 'Applications of NMR Spectroscopy in Organic Chemistry,' Holden-Day, San Francisco, 1966, pp. 49ff.
[14] E. Zissis and N. K. Richtmyer, *J. Org. Chem.*, 1961, **26**, 5244.

for rubropilosin (−67°), pilorubrosin (−66°), and rubropiloside (*ca.* −70°) in methanol support the β-configuration suggested for the present set of compounds.

The third component of the mixture of plant phenols (PR1) could not be isolated in pure form. When this mixture was however saponified as before, it afforded only rubropiloside (IV) as the alcohol component of the ester mixture, but three acids were identified by g.l.c. of their methyl esters, *viz.* cinnamic acid [from (I)], benzoic acid [from (II)], and β-phenylpropionic acid, the latter being a minor component of the mixture of acids. A third ester of rubropiloside was therefore present: that this was its mono-β-phenylpropionate, and not a polyester, was demonstrated by the mass spectrum of the total mixture (PR1) and of its peracetylated and permethylated products. Table 3 lists peaks which are relevant in this regard. In analogy with the structures for rubropilosin (I) and pilorubrosin (II), it is likely that this minor component is the dihydrorubropilosin (III).

TABLE 3

Mixture of glycoside esters			Peracetylated mixture of glycoside esters — *m/e* Values assigned to *			Permethylated mixture of glycoside esters		
DHR	R	P	DHR	R	P	DHR	R	P
434 (M^+)	432 (M^+)	406 (M^+)	421	419	393	504 (M^+)	502 (M^+)	476 (M^+)
295	293	267	133	131	105	337	335	309
133	131	105				336	334	308
						133	131	105

* DHR = Dihydrorubropilosin, R = rubropilosin, P = pilorubrosin, or their peracetates or permethyl ethers respectively.

With the sugar in these glycoside esters established as allose, it was finally possible to account for the unusually low frequency of their ester carbonyl stretching frequencies in potassium bromide dispersions, as against the normal value found [1] for lacticolorin. Dreiding models of lacticolorin show that the hydrogen atom of only the equatorial 4-OH (and not also the equatorial 3-OH) of its glucose system can approach the π-cloud of the phenyl ring of the ester group to hydrogen-bond to it.[15] In the case of the allose esters, however, both the equatorial 4-OH and the axial 3-OH are well placed to hydrogen bond simultaneously in a bifurcate manner for both benzoyl and cinnamoyl substituents on 6-OH. The i.r. spectra of these compounds were therefore run in acetonitrile * solutions (*ca.* 0·7% w/v); the carbonyl stretching absorptions were found at 1722 (lacticolorin, benzoate), 1720 (pilorubrosin, benzoate), and 1715 cm⁻¹ (rubropilosin, cinnamate). The hydroxy-stretching absorptions moved accordingly: in the case of lacticolorin the broad single peak at 3340 cm⁻¹ (KBr) shifted to 3490 cm⁻¹ (MeCN); in the case of rubropilosin well-resolved peaks at 3500 and 3420 (KBr) moved to 3620 and 3540 cm⁻¹ (MeCN), and in the case of pilorubrosin similarly from 3490 and 3420 (KBr) to 3630 and 3550 cm⁻¹ (MeCN). Hydrogen bonding from hydroxy-groups to π-cloud systems is well known; [15] it is striking to note its effect, in the preferred conformations imposed on these allose ester derivatives by such bonding in the solid state, on their ester carbonyl stretching frequencies through inductive withdrawal.

* At the cell path length used (0·1 mm) acetonitrile was transparent over the range 2200—1600 cm⁻¹.

EXPERIMENTAL

Experimental procedures and spectral measurements were carried out as in the preceding paper.

Leaves of *Protea rubropilosa* Beard were collected on Mariepskop (altitude *ca.* 6000 ft) in the Eastern Transvaal in December, air-dried, and milled. The leaf powder (500 mg) on extraction (Soxhlet; 24 h) with methanol gave an extract which on p.c.[16] showed three phenolic spots with Pauly's reagent at R_F 0·85 (PR1), 0·40 (PR2), and 0·06 (PR3); t.l.c. in benzene–methanol–acetic acid (7 : 2 : 1 v/v) showed only two phenolic spots, at R_F 0·58 (PR1) and 0·40 (PR2); t.l.c. in benzene–methanol (4 : 1 v/v) showed a separation of PR1 into two spots at R_F 0·53 and 0·48.

Rubropilosin and Pilorobrusin.—The leaf powder (1 kg) was extracted (Soxhlet; 24 h) with methanol to give a dried extract (315 g). This (80 g) was spread on silica gel (80 g) and chromatographed by eluting with benzene–methanol to give the fractions containing (by t.l.c.) the PR1 mixture together with chlorophyll (9·6 g in all); this mixture was dissolved in warm ethanol (9 ml) and water (18 ml) and allowed to crystallize. The insoluble material was filtered off (3·4 g; hereafter referred to as the glycoside mixture), leached with hot water, and crystallized from 50% aqueous ethanol to yield *rubropilosin* (1·5 g, 0·59%), m.p. 97—100° (Found: C, 58·8; H, 5·8. $C_{22}H_{24}O_9,H_2O$ requires C, 58·7; H, 5·8%), $[\alpha]_D$ −67° (*c* 0·92), $\nu_{max.}$ 3500, 3420, and 3340 (OH), 1685 (C=O), 1625 (C=C olefinic), 1591 and 1510 (arom.), and 1290 and 1046 cm⁻¹ (C–O), *m/e* 432 (M^+, 0·3%), 293, 140, 131 (100%), 123, 103, 85, 77, 73, 57, 44, and 43, δ (100 MHz; C_2D_6SO) 7·02 (1H, d, *S* 8), 6·78 (1H, d, *S* 2), 6·57 (1H, dd, *S* 8 and 2) (arom. aglycone), 7·9—7·3 (5H, m, arom. ester), 7·52 and 6·64 (2H, 2d, *S* 16, *trans* olefinic H of ester), 8·48br (1H, phenolic OH), and 4·98 (1H, d, *S* 8, anomeric H). The aqueous filtrates were extracted with ether and dried *in vacuo*. The residue was dissolved in the minimum volume of boiling water, treated with charcoal, and filtered, and the filtrate was cooled. Some oily material separated and was quickly filtered off. The filtrate slowly deposited crystals which, together with similar material recovered from the oily product and the foregoing ether extract by crystallization from water, on further recrystallization from hot water gave *pilorubrosin* (1·32 g, 0·52%), m.p. 167—169° (Found: C, 58·8; H, 5·5. $C_{20}H_{22}O_9$ requires C, 59·1; H, 5·5%), $[\alpha]_D$ −66° (*c* 0·87), $\nu_{max.}$ 3490 and 3420 (OH), 1683 (C=O), 1600 and 1510 (arom.), 1285 and 1080

[15] L. J. Bellamy, 'Advances in Infrared Group Frequencies,' Methuen, London, 1969, p. 246.
[16] P. E. J. Kruger and G. W. Perold, *J. Chem. Soc. (C)*, 1970, 2131.

(C–O), and 712 cm^{-1} (C_6H_5), *m/e* 406 (M^+, 0·6%), 267, 249, 140, 123, 105 (100%), 85, 77, 73, 57, and 43, δ (100 MHz; C_2D_6SO) 7·02 (1H, d, *S* 8), 6·77 (1H, d, *S* 2), and 6·51 (1H, dd, *S* 8 and 2) (arom. aglycone), 8·01 (2H, dd, *S* 8 and 2) and 7·7—7·4 (3H, m) (arom. ester), and 5·0 (1H, d, *S* 7, anomeric proton), δ (100 MHz; CD_3OD) 7·13, 6·87, and 6·60, 8·08 and 7·7—7·4, and 5·14 (in the same order as before). Both rubropilosin and pilorubrosin were acetylated (for mass spectral purposes only) by keeping them (8 mg each) in pyridine (0·1 ml) and acetic anhydride (0·1 ml) for 16 h and then evaporating the product: neither sample showed a parent peak and the major peaks for rubropilosin peracetate were at *m/e* 419, 257, 169, 131 (100%), 109, 103, and 43, and for pilorubrosin peracetate at *m/e* 393, 231, 169, 109, 105 (100%), 77, and 43.

Rubropilosin penta-O-methyl ether was obtained from rubropilosin (1 g) with methyl iodide (5 ml) and silver oxide (2·7 g) in dimethylformamide (5 ml) [17] as a syrup (1·2 g) which after chromatography in benzene–ethyl acetate was chromatographically pure but non-crystalline (Found: C, 64·0; H, 6·8. $C_{27}H_{34}O_9$ requires C, 64·5; H, 6·8%), $\nu_{max.}$ ($CHCl_3$) 1704 (C=O), 1631 (C=C, olefinic), 1590 and 1505 (arom.), 1265, 1155, 1095, and 1030 cm^{-1}, *m/e* 502 (M^+, 2%), 335, 334, 155, 131 (100%), 103, 101, 88, 77, 75, 71, and 45.

Pilorubrosin penta-O-methyl ether (1·23 g) was similarly prepared from pilorubrosin (1·02 g) and obtained as a chromatographically pure syrup (Found: C, 62·8; H, 6·8. $C_{25}H_{32}O_9$ requires C, 63·0; H, 6·8%), $\nu_{max.}$ ($CHCl_3$) 1720 (C=O), 1600 and 1510 (arom.), 1280, 1205, 1100, 1050, and 1000 cm^{-1}, *m/e* 476 (M^+, 2%), 309, 308, 155, 105 (100%), 101, 88, 77, 75, 71, and 45.

Alkaline Hydrolysis of Rubropilosin and Pilorubrosin.—Rubropilosin (420 mg) was refluxed with barium hydroxide octahydrate (700 mg) in water (50 ml) for 2 h; the solution was made acid (Congo Red) with N-sulphuric acid and filtered. The filtrate was extracted continuously (16 h) with ether to yield a solid (149 mg). This (10 mg) was methylated in ether (1 ml) with excess of 0·2M-diazomethane in ether and the total product, which showed only one spot at R_F 0·37 (as for methyl cinnamate) on t.l.c. in benzene, was analysed by g.l.c. (10% FFAP on GasChrom Q, 200°, carrier gas at 30 ml min^{-1}) when a peak at a retention time of 5·07 min appeared, again as for methyl cinnamate.

The foregoing solid product, crystallized from water and sublimed, was cinnamic acid, m.p. 131—133° (sealed tube) (Found: C, 72·7; H, 5·4. Calc. for $C_9H_8O_2$: C, 73·0; H, 5·4%), identical with an authentic sample (mixed m.p. and i.r. spectrum). The foregoing acidified filtrate was neutralized with barium carbonate, filtered, and evaporated *in vacuo* to give a residue (302 mg) which crystallized (188 mg) from hot water to give *rubropiloside* (IV), m.p. 184—185° (Found: C, 49·3; H, 6·3. $C_{13}H_{18}O_8,H_2O$ requires C, 48·75; H, 6·3%), $[\alpha]_D$ −67° (*c* 0·65), $\nu_{max.}$ 3350br (OH), 1615, 1595, and 1510 (arom.), 1285, 1205, 1085, 1037, and 827 cm^{-1}.

The alkaline hydrolysis of pilorubrosin (484 mg) was carried out as for rubropilosin and afforded an acidic extract (147 mg, dried) shown to be benzoic acid, m.p. 120·5—121·5° (sealed tube) from water (Found: C, 69·2; H, 5·0. Calc. for $C_7H_6O_2$: C, 68·9; H, 4·95%), by mixed m.p. and i.r. spectrum, and rubropiloside (226 mg from 369 mg of crude product), m.p. 185—186° (from hot water) (Found: C, 48·8; H, 6·35%), $[\alpha]_D$ −73° (*c* 0·71), identical with the earlier sample (mixed m.p. and i.r.).

Alkaline Hydrolysis of the Glycoside Mixture.—The glycoside mixture (432 mg, as obtained from the leaves) in the same manner gave an acidic extract (130 mg, solid), which (10 mg) was methylated with diazomethane as before; the ester mixture on t.l.c. in benzene showed spots at R_F 0·37 (for methyl cinnamate) and 0·47 (for methyl benzoate). G.l.c. (carried out as before) showed peaks at retention times of 1·28 (major), 2·42 (minor), and 5·07 min (major), identical with those for methyl benzoate, β-phenylpropionate, and cinnamate, respectively, when these were run under the same conditions. The neutralized aqueous solution, worked up as before, afforded rubropiloside, m.p. 182—184°, $[\alpha]_D$ −73° (*c* 0·75), $\nu_{max.}$ as before.

Rubropiloside hexa-acetate was prepared as before for mass spectroscopy only and exhibited *m/e* 554 (M^+, <0·1%), 331, 169, 127, 109, and 43 (100%). Rubropiloside (102 mg) was methylated [17] with methyl iodide (3 ml) and silver oxide (0·5 g) in dimethylformamide (3 ml) as before and gave rubropiloside hexa-*O*-methyl ether as a chromatographically pure syrup (41 mg from 127 mg crude product) which very gradually crystallized and had a mass spectrum [*m/e* 386 (M^+, 2%), 219, 218, 187, 155, 116, 111, 101 (100%), 99, 89, 88, 75, 73, 71, and 45] which was, apart from the different molecular ion, closely similar to that of phenyl tetra-*O*-methyl-β-D-glucopyranoside.

Degradation of Rubropiloside.—Rubropiloside (500 mg) in water (5 ml), ethanol (75 ml), and 10M-hydrochloric acid (0·2 ml) was hydrogenolysed over 10% palladium–carbon (250 mg) for 40 min at 1 Torr. The filtered solution was neutralized with Dowex 1-X8 resin (carbonate form) and evaporated to a residue (390 mg), showing only one spot at R_F 0·35 on t.l.c. in benzene–methanol–acetic acid (7 : 2 : 1 v/v); *cf.* R_F 0·18 for rubropiloside. It was treated in methanol (2·5 ml) with 0·2M-diazomethane in ether (25 ml) for 16 h to give a product (453 mg) showing no phenolic reaction with Pauly's reagent. This was refluxed in N-sulphuric acid (35 ml) for 6 h and the solution extracted with ether to give 4-hydroxy-3-methoxytoluene (165 mg) which distilled (112 mg) at 55—60° and 0·06 Torr and was characterized by conversion (112 mg) into its benzoate (Schotten–Baumann procedure) (90 mg), m.p. 72—73° (from ethanol) (Found: C, 74·2; H, 5·8. Calc. for $C_{15}H_{14}O_3$: C, 74·4; H, 5·8%), identical (i.r. and mixed m.p.) with an authentic sample of 4-benzoyloxy-3-methoxytoluene. In a separate sequence, rubropiloside (1·61 g) was hydrogenolysed as before. The product (1·31 g) was hydrolysed (6 h) in boiling N-sulphuric acid (40 ml) and extracted with ether; the aqueous solution was neutralized with barium carbonate, filtered, and evaporated *in vacuo*. Part (650 mg) of the residue (866 mg) was shaken in pyridine (6 ml) and acetic anhydride (10 ml) for 5 h and left for 16 h to give the crude sugar acetate as a syrup (1·1 g). This was chromatographed in benzene–ethyl acetate (3 : 1 v/v) to give the chromatographically pure sugar acetate (618 mg), which was deacetylated in methanol (6 ml) with sodium methoxide [sodium (0·01 g) in methanol (2 ml)] for 20 min. The solution was neutralized with Amberlite IR-120 resin (acid form), filtered, and evaporated *in vacuo* to a syrup (261 mg), which gave crystals (134 mg; from hot methanol) of β-D-allose, m.p. 130—132° (Found: C, 39·8; H, 6·9. Calc. for $C_6H_{12}O_6$: C, 40·0; H, 6·7%), $[\alpha]_D$ +14·5° (equilibr., *c* 1·11 in H_2O), $\nu_{max.}$ 3490, 3330, 3130, 2970, 2910, 2890, 2870, 1426, 1399, 1370, 1335, 1278, 1146, 1130, 1090, 1070, 1050, 1040, 896, and 724 cm^{-1}, identical (i.r. and mixed m.p.) with an

[17] R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, 1955, **67**, 32.

authentic sample, and with a synthetic sample here prepared[9] from (−)-D-ribose. The pure sugar (70 mg) in water (1·4 ml) was warmed at 50—60° for 7 min with *p*-bromophenylhydrazine (70 mg) in ethanol (1·4 ml). The solution was evaporated after 16 h; the residue was triturated with ether and water and crystallized (33 mg, crude) from hot water to give the *p*-bromophenylhydrazone of β-D-allose, m.p. 144—146° (lit.,[8] 145—147°) (Found: C, 41·4; H, 5·0; N, 8·0. Calc. for $C_{12}H_{17}BrN_2O_5$: C, 41·3; H, 4·9; N, 8·0%), identical (i.r. and mixed m.p.) with a sample prepared from authentic β-D-allose. The osazone of the sugar (20 mg) was prepared by heating in water (0·2 ml) with phenylhydrazine (0·04 ml) in acetic acid (0·04 ml) and water (0·1 ml) for 1 h at 95°; it was recrystallized from aqueous ethanol to m.p. 162—163° (lit.,[10] 162—163°) (Found: C, 60·1; H, 6·15; N, 15·75. Calc. for $C_{18}H_{22}N_4O_4$: C, 60·3; H, 6·2; N, 15·6%), identical (i.r. and mixed m.p.) with an authentic sample prepared in the same manner. The pure sugar (1 mg) in Tri-sil 'Z' (0·1 ml) was warmed at 60—70° for 2 min and the solution was analysed by g.l.c. (5% GE SE-52 on Chromosorb W, helium at 30 ml min^{-1}), together with similar solutions prepared from authentic and synthetic β-D-allose; all samples showed only one * peak at a retention time of 6·9 min (*cf.* 8·3 min for α-glucose, *i.e.* $R_{\alpha-g}$ 0·83) at 200°, and of 16·4 min ($R_{\alpha-g}$ 0·80) at 180° (lit.,[18] $R_{\alpha-g}$ 0·81 at 140°).

*Degradation of Rubropilosin Penta-*O*-methyl Ether.*—Rubropilosin penta-*O*-methyl ether (V) (466 mg) in methanol (10 ml) was refluxed with barium hydroxide octahydrate (500 mg) in water (20 ml) for 2 h. The solution was extracted continuously (17 h) with ether to yield penta-*O*-methylrubropiloside (VI) (348 mg), R_F 0·12 in benzene–ethyl acetate (2 : 1 v/v) [*cf.* R_F 0·48 for the starting material (V)] then acidified with hydrogen chloride gas and again extracted continuously (16 h) to afford cinnamic acid (136 mg crude), m.p. 131·5—133° (sealed tube) from aqueous ethanol (Found: C, 72·95; H, 5·4. Calc. for $C_8H_8O_2$: C, 73·0; H, 5·4%), identical with an authentic sample (i.r. and mixed m.p.). The penta-*O*-methylrubropiloside (348 mg), in 96% ethanol (50 ml) and 10M-hydrochloric acid (0·5 ml) was hydrogenolysed over 10% palladium–carbon (200 mg) for 1 h at 1 Torr, and the syrupy product (340 mg) was refluxed in methanol (10 ml) with N-sulphuric acid (40 ml) for 6 h. The acidic solution was neutralized with barium carbonate, filtered to remove the aglycone together with barium sulphate, and evaporated *in vacuo* at 50°. The residue was extracted with warm acetone to give a product (280 mg) which was chromatographed in benzene–methanol to give chromatographically pure (+)-2,3,4-*tri-*O*-methyl-*D*-allose* (VIII) (134 mg), m.p. 106·5—108° (from benzene) (Found: C, 48·7; H, 8·3. $C_9H_{18}O_6$ requires C, 48·6; H, 8·2%), $[\alpha]_D$ +25° (*c* 0·4), $\nu_{max.}$ 3350br, 2960, 2924, 2890, 2830, 1459, 1358, 1310, 1230, 1205, 1123, 1101, 1088, 1059, 1040, 999, 937, 913, 719, and 632 cm^{-1}.

*Degradation of Pilorubrosin Penta-*O*-methyl Ether.*—Pilorubrosin penta-*O*-methyl ether (606 mg) was similarly hydrolysed in boiling barium hydroxide solution to afford penta-*O*-methylrubropiloside (VI) (444 mg) and the acid portion (173 mg); the latter gave benzoic acid, m.p. 121—122° (Found: C, 69·0; H, 4·9. Calc. for $C_7H_6O_2$: C, 68·9; H, 4·95%), identified by i.r., mixed m.p., and g.l.c. of its methyl ester as before. The penta-*O*-methylrubropiloside (VI) was hydrogenolysed, and then hydrolysed in sulphuric acid solution, as before; the reduced aglycone was then extracted (1·5 h) with ether (114 mg product), distilled at 60—65° and 0·15 Torr (74 mg), and benzoylated (Schotten–Baumann) to give 4-benzoyloxy-3-methoxytoluene (113 mg), m.p. 75—76° (Found: C, 74·2; H, 5·9. Calc. for $C_{15}H_{14}O_3$: C, 74·4; H, 5·8%), identical (i.r. and mixed m.p.) with a reference sample. The sugar portion was recovered as before (246 mg) and chromatographed to give (+)-2,3,4-tri-*O*-methyl-D-allose (VIII) (146 mg), m.p. 107—109° (Found: C, 49·0; H, 8·3. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2%), identical with the previous sample (i.r. and mixed m.p.).

Proof of Identity of (+)-2,3,4-*Tri-*O*-methyl-*D*-allose.*—The tri-*O*-methylallose (1 mg) was kept in 0·25M-sodium periodate solution (0·12 ml) for 1 h at 0°. Ethylene glycol (*ca.* 3 mg) was added and after 5 min at room temperature the solution was neutralized (phenolphthalein) with 0·5N-sodium hydroxide, evaporated *in vacuo*, and extracted † with warm acetone. The acetone extract was concentrated to *ca.* 0·1 ml and samples (10 μl) were spotted on precoated silica gel plates (Merck DC-Fertigplatten Kieselgel F_{254}). The plates were run in benzene–methanol (9 : 1 v/v), and sprayed with aniline phthalate reagent: the product was unchanged starting material (reddish-brown spot at R_F 0·17). The tri-*O*-methylallose (25 mg) was next kept with sodium borohydride (10 mg) in water (0·2 ml) for 1 h; acetic acid was added to remove excess of borohydride, the solution was evaporated, and the tri-*O*-methylallitol was extracted with acetone. Authentic 2,3,4- and 2,3,6-tri-*O*-methyl-D-glucose were in the same way reduced to 2,3,4- and 2,3,6-tri-*O*-methyl-D-sorbitol, respectively. These three glycitols (1 mg each) were then oxidized with sodium periodate and the oxidation products were run on t.l.c. as before. The results are in Table 4.

TABLE 4

Substrate oxidized	Product	Type of product	Colour of t.l.c. spot	R_F [replicate values in benzene–methanol (9 : 1 v/v)]
2,3,4-Tri-*O*-methyl-D-allose	2,3,4-Tri-*O*-methyl-D-allose (VIII)	Hexaose	Reddish brown	0·17, 0·17
2,3,4-Tri-*O*-methyl-D-allitol	2,3,4-Tri-*O*-methyl-L-ribose (XX)	Pentaose	Plum	0·35, 0·35, 0·35, 0·35
2,3,4-Tri-*O*-methyl-D-sorbitol	2,3,4-Tri-*O*-methyl-L-xylose (XXI)	Pentaose	Plum	0·39, 0·39, 0·38, 0·38
2,3,6-Tri-*O*-methyl-D-sorbitol	2,3-Di-*O*-methyl-L-threose (XXII)	Tetraose	Pale beige	0·49, 0·49

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* No anomeric peaks, as found for α- and β-glucose,[1] appeared in the case of β-D-allose.

† The R_F values of the products were considerably affected by the salts present if the aqueous solution was used as such for t.l.c.

[18] C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, 1963, **85**, 2497.

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