Metabolites of Proteaceae. Part VII.[1] Lacticolorin, a Phenolic Glucoside Ester, and Other Metabolites of *Protea lacticolor* Salisb.

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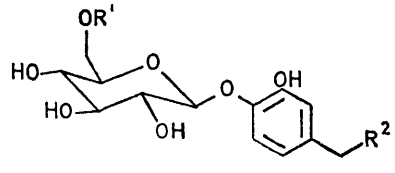
Lacticolorin, from the leaves of *Protea lacticolor* Salisb., is shown to be 2-hydroxy-4-hydroxymethylphenyl 6-*O*-benzoyl-β-D-glucopyranoside. It is accompanied in the plant by benzoic, *p*-hydroxybenzoic, vanillic, and protocatechuic acids, as well as by 3,4-dihydroxybenzyl alcohol.

THE genera *Leucadendron* and *Leucospermum* of the family Proteaceae have been found to contain, respectively, leucodrin [2] and diastereoisomeric analogues of it (conocarpin,[3] reflexin and conocarpic acid [4]). These metabolites may be considered as *C*-glycosyl compounds [5] arising by Michael condensation [6] of *p*-coumaric acid and L-galactono-γ-lactone (or their biogenetic equivalents).[1] The Proteaceae have also been shown to contain the glycoside arbutin (*p*-hydroxyphenyl β-D-glucoside) by paper chromatographic screening [7] and by isolation of the compound.[8] A study of *Protea lacticolor* Salisb. has now shown that it contains a substituted phenyl glucoside benzoate as a major leaf constituent; this metabolite, lacticolorin, is shown to be **2**-hydroxy-**4**-hydroxymethylphenyl **6**-*O*-benzoyl-β-D-glucopyranoside (I).

Other constituents found in the leaves of *P. lacticolor* were benzoic, *p*-hydroxybenzoic, vanillic, and protocatechuic acids, and **3,4**-dihydroxybenzyl alcohol (see Experimental section); the last three of these metabolites thus form a coherent group together with lacticolorin, the common factor being **3,4**-dihydroxybenzyl alcohol. That this compound is available in the plant as the free alcohol, and also that it is easily etherified, was demonstrated by extracting the leaves with methanol, when the benzyl methyl ether was obtained,



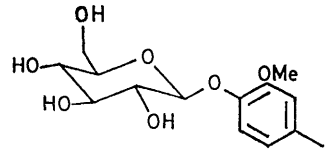
(I) R^1 = Bz , R^2 = OH
(II) R^1 = H , R^2 = OH
(III) R^1 = R^2 = H

and with ethanol, when the product isolated was the benzyl ethyl ether (see Experimental section).

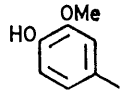
Lacticolorin, $C_{20}H_{22}O_9$, m.p. **192—195°**, $[\alpha]_D$ **−58°**, is a hydroxy-ester (I), its i.r. spectrum showing aryl ester stretching absorption [9] at **1720** cm^{-1}. Five hydroxy-groups were shown to be present by the formation of a penta-acetate and a penta-*O*-methyl ether.

[1] Part VI, G. W. Perold, A. J. Hodgkinson, A. S. Howard, and P. E. J. Kruger, *J.C.S. Perkin I*, 1972, 2457.
[2] W. S. Rapson, *J. Chem. Soc.*, 1938, 286; G. W. Perold and K. G. R. Pachler, *J. Chem. Soc.* (*C*), 1966, 1923.
[3] P. E. J. Kruger and G. W. Perold, *J. Chem. Soc.* (*C*), 1970, 2127.
[4] G. W. Perold, A. J. Hodgkinson, and A. S. Howard, *J.C.S. Perkin I*, 1972, 2450.
[5] G. O. Aspinall in ' Chemistry of Carbon Compounds,' ed. E. H. Rodd, Elsevier, Amsterdam, 1962, vol. V, p. 171.
[6] R. D. Diamand and D. Rogers, *Proc. Chem. Soc.*, **1964**, 63.
[7] M. C. B. van Rheede van Oudtshoorn, *Planta Med.*, 1963, **11**, 399.
[8] Unpublished results from our laboratory.
[9] L. J. Bellamy, ' The Infrared Spectra of Complex Molecules,' 2nd edn., Methuen, London, 1962, p. 179.

On saponification with barium hydroxide solution it afforded benzoic acid and the glycoside, debenzoyl-lacticolorin (II). Acidic hydrolysis of lacticolorin yielded benzoic acid and the sugar portion, but the phenolic aglycone was too sensitive to survive this treatment. Debenzoyl-lacticolorin could however readily be catalytically hydrogenolysed to the glycoside (III), and on subsequent acidic hydrolysis afforded 3,4-dihydroxytoluene. The position of attachment of the sugar to the phenolic aglycone was determined by converting hydrogenolysed debenzoyl-lacticolorin (III) into its aryl methyl ether (IV) before hydrolysis in acid medium. The product then obtained was 4-hydroxy-3-methoxytoluene (V).

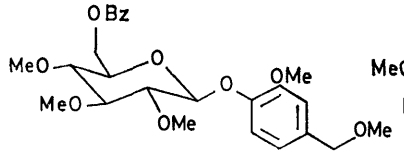


(IV)

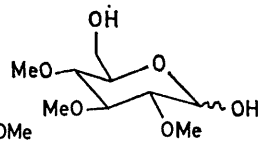


(V)

The elucidation of the full structure of lacticolorin therefore required demonstration of the nature of its sugar component and its ring size, and the position of attachment of the ester group: this was effected by degradation of lacticolorin penta-*O*-methyl ether (VI). The mode of linkage of the aglycone to the sugar was also demonstrated (see later).

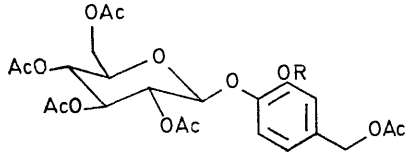


(VI)



(VII)

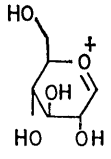
Hydrolysis and hydrogenolysis of the ether (VI) afforded benzoic acid, 4-hydroxy-3-methoxytoluene



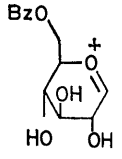
(X) R = Ac
(XI) R = Me

(V), and a tri-*O*-methylglucose (VII), which was shown to be 2,3,4-tri-*O*-methyl-D-glucose by its i.r. spectrum,[10] its optical rotation, its behaviour on g.l.c. analysis of its trimethylsilyl derivative, and direct comparison with a synthetic sample. If no acyl migration and no furanose–pyranose ring interconversions took place during the preparation of the penta-*O*-methyl ether (VI), then this finding in itself suffices to establish the structure of lacticolorin. Acyl group migrations in sugar esters have been reported[11] to occur under the influence of lead subacetate at elevated temperatures, but not to occur at room temperature. As lacticolorin was here methylated to give the ether (VI) in the presence of silver oxide at room temperature, acyl group migration may in this case be discounted. Similarly, furanose–pyranose interconversions proceed *via* the ring-opened free sugar component; as only the one debenzoyl-lacticolorin was obtained in all our experiments, ring isomerization during the permethylation step is unlikely.

The mass spectrum of lacticolorin itself, however, brought direct support for the location of the ester group in the sugar portion of the native compound. Methyl glucopyranosides have been shown[12] to fragment in the ion source to yield the glucose oxonium

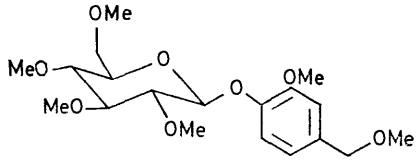


(VIII)



(IX)

ion (VIII) ($M - OCH_3$, *m/e* 163, 8% intensity relative to the base peak at *m/e* 60). In the case of lacticolorin, no peak was found for this ion, but a relatively strong peak was observed at *m/e* 267 ($M - C_7H_7O_3$, intensity 15% of base peak at *m/e* 105) for an ion, such as (IX), resulting from the analogous glycosidic ether fragmentation. The ester group is not therefore in the aglycone of lacticolorin (as it is, for example, in the case of salireposide).[13] The same conclusion is drawn from the full analysis, reported elsewhere,[14] of the mass spectra of lacticolorin penta-*O*-methyl ether and lacticolorin penta-acetate; the latter furthermore shows a relatively



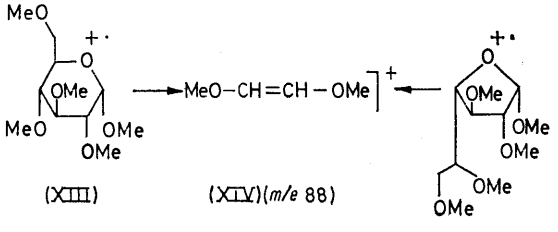
(XII)

abundant (28%) ion at *m/e* 231, indicative of three vicinal acetoxy-groups and so indicating the 6-position[15] on the sugar for the benzoate group.

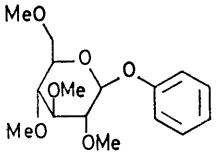
The pyranose structure for the sugar is indicated by the mass spectra (available elsewhere[14]) of debenzoyl-lacticolorin hexa-acetate (X) and debenzoyl-lacticolorin

[10] I. A. Pearl, S. F. Darling, and L. Sell, *Tappi*, 1962, **45**, 808.
[11] I. A. Pearl and S. F. Darling, *Arch. Biochem. Biophys.*, 1963, **102**, 33.
[12] P. A. Finan, R. I. Reed, W. Snedden, and J. M. Wilson, *J. Chem. Soc.*, 1963, 5945.
[13] I. A. Pearl and S. F. Darling, *Phytochem.*, 1968, **7**, 821.
[14] P. Beylis, Ph.D. Thesis, University of the Witwatersrand, Johannesburg, 1971.
[15] I. A. Pearl and S. F. Darling, *Phytochem.*, 1968, **7**, 831.

aryl methyl ether penta-acetate (XI) (see Experimental section) in relation to the known [16,17] behaviour of the penta-acetates of glycopyranosides and glycofuranosides in the ion source. The most direct mass spectral evidence is, however, provided by the mass spectrum of debenzoyl-lacticolorin hexa-*O*-methyl ether (XII) with reference to the known [18-21] differences in mass spectral cleavages of pyranosides and furanosides. These differences are due [18,21] to the preferred [22] cleavage of the pyranoside form (XIII), relative to the furanoside form (XV) of a permethylated hexose to afford the common ion (XIV), whose main contributor is the C-2,C-3 unit of these sugar permethyl ethers. Thus in the case of the methyl pyranoside (XIII) this ion is the base peak, while for the methylfuranoside (XV) it is formed in only 10% abundance relative to the base peak (here at *m/e* 101). The relative abundance of this ion (XIV) is reduced to 25% in passing from the permethyl pyranoside (XIII) to the phenyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside [18] (XVI), so that a relative abundance of *ca.* 2% might be expected for this peak in the case of the corresponding phenyl



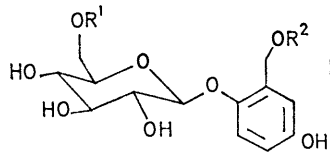
(XIII) (XIV) (*m/e* 88) (XV)



(XVI)

2,3,4,6-tetra-*O*-methyl-D-furanoside. The mass spectra of debenzoyl-lacticolorin hexa-*O*-methyl ether (XII) and its phenyl analogue (XVI) were indeed closely similar and showed this ion at *m/e* 88 at relative abundances of 27% for both compounds [(XII) and (XVI)].

Finally, the configuration at C-1 in lacticolorin was determined from the n.m.r. characteristics of H-1.[23-25] The anomeric proton in lacticolorin appeared (in hexadeuteriodimethyl sulphoxide solution) as a doublet (separation 8 Hz) centred at δ 4·98; H-1 is therefore [24,25] axial to the pyranose ring and the configuration of C-1 in lacticolorin is therefore β. This was supported by the o.r.d. behaviour of lacticolorin in methanol solution: over the range 220—350 nm its o.r.d. curve paralleled that of phenyl β-D-glucopyranoside ($[\alpha]_D$ −72°), which was in turn a mirror image of that of phenyl α-D-glucopyranoside ($[\alpha]_D$ +181°).* Hudson's isorotation rules [26]



(XVII) R^1 = H, R^2 = Bz (XIX) R^1 = Bz, R^2 = H
(XVIII) R^1 = Bz, R^2 = H (XX) R^1 = H, R^2 = Bz

therefore are followed in these cases. Lacticolorin (I) from *Protea lacticolor* is therefore a positional isomer of salireposide [27] (XVII) and nigracin [28] (XVIII), and is related to populin [29] (XIX) and tremuloidin [30] (XX), found in *Populus* and *Salix* species.

EXPERIMENTAL

M.p.s were taken on a Kofler micro hot-stage apparatus. Spectrophotometers used were Perkin-Elmer 521 (i.r., for potassium bromide dispersions or neat liquid films), JASCO ORD/UV/CD-5 (o.r.d., for methanolic solutions), A.E.I. MS-9 (mass spectra; only peaks of more than 5% relative abundance are quoted unless shown otherwise), and Varian HA-100 or Hitachi–Perkin-Elmer R20A [n.m.r.; only protons showing clear signals are assigned, and chemical shift values (in p.p.m. relative to tetramethylsilane), and separations (*S* in Hz) are read from the spectra]. Optical rotations were measured in methanolic solution on a Perkin-Elmer 141 polarimeter. G.l.c. analyses were carried out on a Perkin-Elmer 880 instrument. T.l.c., unless shown otherwise, was performed on plates of silica gel GF_{254} (nach Stahl); phenolic spots were made visible with Pauly's reagent,[31] reducing sugar spots with aniline phthalate solution,[32] and other spots with either chromic acid [33] or sulphuric acid solutions. Paper chromatography (p.c.) was carried out in n-butanol–toluene (1 : 1 v/v) on paper impregnated with glycerol.[34] Column chromatography was performed over silica gel (Merck, 0·05—0·2 mm).

* Turning points on the o.r.d. curves were found (deg./nm) for phenyl α-D-glucopyranoside at +23/258, +27/262, +15/266, +38/269, +23/272, and +39/275; for phenyl β-D-glucopyranoside at −3/257, −16/263, −7/266, −22/270, −16/273, and −31/278; and for lacticolorin at +9/264 and −31/286.

[16] K. Biemann, D. C. de Jongh, and H. K. Schnoess, *J. Amer. Chem. Soc.*, 1963, **85**, 1763.
[17] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectroscopy,' vol. II, Holden-Day, San Francisco, 1964, pp. 204ff.
[18] N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, and B. M. Zolotarer, *Tetrahedron*, 1963, **19**, 2209.
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[20] K. Heyns, D. Müller, R. Stute, and H. Paulsen, *Chem. Ber.*, 1967, **100**, 2664.
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[22] Ref. 21, p. 63.
[23] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, 1958, **80**, 6098.
[24] J. M. van der Veen, *J. Org. Chem.*, 1963, **28**, 564.
[25] B. Capon and D. Thacker, *Proc. Chem. Soc.*, 1964, 369.
[26] C. S. Hudson, *Adv. Carbohydrate Chem.*, 1948, **3**, 15.
[27] H. Thieme, *Pharmazie*, 1966, **21**, 769.
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[29] N. K. Richtmyer and E. Yeakel, *J. Amer. Chem. Soc.*, 1934, **56**, 2495.
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[31] 'Chromatography with Particular Consideration of Paper Chromatography,' E. Merck AG, Darmstadt, 2nd edn., p. 144.
[32] S. M. Partridge, *Nature*, 1949, **164**, 443.
[33] H. Ertel and L. Horner, *J. Chromatog.*, 1962, **7**, 268.
[34] Ref. 3, p. 2131.

Reactions were at room temperature unless specified otherwise.

Milled air-dried leaves (1 g) of *Protea lacticolor* Salisb. were extracted with methanol; p.c. of the extract [34] showed a strong phenolic spot at R_F 0·86, with weaker spots at R_F 0·71 and 0·57; t.l.c. showed six phenolic spots, designated as PL1, 2, *etc.* in increasing order of polarity.

The leaf powder (1 kg) was extracted (Soxhlet) for 24 h with methanol, the extract was concentrated to 1·5 l *in vacuo* at 70°, and methanol was then distilled off while the volume was maintained by adding water. The aqueous solution was made alkaline to litmus with sodium hydrogen carbonate and the clear supernatant was continuously extracted with ether for a total of 423 h to yield 52 g of phenolic constituents and chlorophyll (extract A, 27 g after 6 h, contained PL1 and chlorophyll; extract B, 13·5 g after 95 h, contained phenols PL1—5), worked up as shown later. The aqueous solution was then carefully acidified with hydrogen chloride gas (to avoid dilution) and again extracted continuously with ether to give acid components (extract C, 42 g after 70 h; extract D, 16 g after 293 h). Extract C on t.l.c. showed one non-phenolic and three phenolic acid spots at R_F 0·76, 0·57, 0·28, and 0·14 in benzene–acetic acid (9 : 1 v/v).

Chromatography of extract C (20 g) over silica gel (600 g), with t.l.c. monitoring of the eluted fractions, afforded (*a*) benzoic acid and vanillic acids together; these were readily separated by fractional sublimation [benzoic acid, 0·27% yield based on dried leaf material, m.p. 122—123° (Found: C, 68·85; H, 5·0. Calc. for $C_7H_6O_2$: C, 68·9; H, 4·95%); vanillic acid, 0·08%, m.p. 204—207° (Found: C, 57·1; H, 4·8. Calc. for $C_8H_8O_4$: C, 57·15; H, 4·8%)]; (*b*) *p*-hydroxybenzoic acid, 0·4%, m.p. 214—216° (Found: C, 60·7; H, 4·4. Calc. for $C_7H_6O_3$: C, 60·9; H, 4·4%); (*c*) protocatechuic acid, 3·8%, m.p. 202—203° (Found: C, 54·6, 54·9; H, 3·9, 3·8. Calc. for $C_7H_6O_4$: C, 54·6; H, 3·9%). All four acids were identical with reference compounds (mixed m.p. and i.r. spectra).

Methyl 3,4-*Dihydroxybenzyl Ether*.—The phenolic extract A (27 g) in 50% methanol (230 ml) was continuously extracted (31 h) with light petroleum (b.p. 60—80°) to remove chlorophyll and non-phenolic constituents (11·8 g). Methanol was distilled from the solution and replaced by water and this aqueous solution was continuously extracted with ether (extract E, 5·4 g after 1 h; extract F, 3·8 g after 20 h). Extracts E and F showed only the spot for PL1 at R_F 0·89 on t.l.c. in benzene–methanol–acetic acid (7 : 2 : 1 v/v); PL1 could not be distilled without change and was shown to be *methyl* 3,4-*dihydroxybenzyl ether*. The ether (1·05 g of extract E) was left in pyridine (10 ml) and acetic anhydride (20 ml) for 16 h and the resulting syrup (1·5 g) chromatographed to give *methyl* 3,4-*diacetoxybenzyl ether* (690 mg), liquid, b.p. 130—140° at 0·2 Torr (Found: C, 60·4; H, 5·9. $C_{12}H_{14}O_5$ requires C 60·5; H, 6·1%), R_F 0·5 in benzene–ethyl acetate (9 : 1 v/v), $\nu_{max.}$ 2970, 2930, 2820 (C–H); 1770 (C=O); 1598, 1508, 1428, 1373, 1260, 1225, 1180, 1110, 1014, and 898 cm^{-1}, *m/e* 238 (M^+), δ ($CDCl_3$) 7·18 (5H, arom.), 4·43 (2H, s), 3·37 (3H, s, OCH_3), and 2·25 (6H, s, acetate). The ether (380 mg of extract E) was also kept in methanol (1 ml) and 0·2M-diazomethane solution in ether (20 ml) for 16 h. The recovered material was chromatographed (166 mg) and distilled (90—100° at 0·2 Torr) to give *methyl* 3,4-*dimethoxybenzyl ether* (Found: C, 65·7; H, 7·8. $C_{10}H_{14}O_3$ requires C, 65·9; H, 7·7%), R_F 0·49 in benzene–ethyl acetate (9 : 1 v/v), $\nu_{max.}$ 2930, 2830, 1608, 1592, 1511, 1464, 1418, 1260, 1235, 1158, 1138, 1095, 1030, 854, 808, and 765 cm^{-1}, *m/e* 182 (M^+), δ ($CDCl_3$) 6·87 (3H, m, arom.), 4·40 (2H, s), 3·88 and 3·86 (2 × 3H, s, $ArOCH_3$), and 3·37 (3H, s, OCH_3).

The dihydroxy-ether (600 mg of extract E) was furthermore hydrogenolysed in 96% ethanol (50 ml) and 10M-hydrochloric acid (0·2 ml) over 10% palladium–carbon (300 mg) at 1 Torr for 1 h. The solution was diluted with water (50 ml), neutralized with sodium hydrogen carbonate, filtered, freed of ethanol, and extracted with ether to give a product which on chromatography gave 3,4-dihydroxytoluene (74 mg), m.p. 64—66° (sealed tube) (Found: C, 67·9; H, 6·6. Calc. for $C_7H_8O_2$: C, 67·7; H, 6·5%), identical with an authentic sample (mixed m.p. and i.r. spectra).

Ethyl 3,4-*Dihydroxybenzyl Ether from* P. lacticolor.—The leaf powder (14 g) was extracted (Soxhlet) with 96% ethanol for 17 h. The dried extract (6·1 g) was chromatographed and fractions containing 'PL1' were combined, dried, taken up in water (50 ml), and made alkaline with sodium hydrogen carbonate. This solution was extracted with light petroleum (2 × 20 ml) and then continuously extracted with ether (1·5 h) to yield crude 'PL1' (150 mg). This (150 mg) was kept in pyridine (2 ml) and acetic anhydride (3 ml) for 16 h and the syrup (195 mg) obtained on evaporation was chromatographed to yield *ethyl* 3,4-*diacetoxybenzyl ether*, liquid, b.p. 120—130° at 0·07 Torr, R_F 0·42 in benzene–ethyl acetate (9 : 1 v/v), δ ($CDCl_3$) 7·12 (3H, arom.), 4·45 (2H, s, $ArCH_2O$), 3·50 (2H, q, *S* 7, $CH_2{\cdot}CH_3$), 2·24 (6H, s, acetate), and 1·20 (3H, t, *S* 7, $CH_2{\cdot}CH_3$).

Lacticolorin.—Ether extract B of the methanol extraction (4·3 g) was chromatographed and the dried fractions containing mainly phenol PL3 (1·05 g) were treated in ethyl acetate solution with ether to afford *lacticolorin* (600 mg) as a powder showing only one spot at R_F 0·47 in benzene–methanol–acetic acid (7 : 2 : 1 v/v). By working up the other fractions containing lacticolorin in this manner the product was obtained in 0·39% yield in all. It crystallized from water with difficulty and the m.p. varied in the range 190—205° for different preparations: a typical m.p. was 192—195° (Found: C, 58·8; H, 5·4. $C_{20}H_{22}O_9$ requires C, 59·1; H, 5·5%), $[\alpha]_D$ −58° *c* (0·59 in MeOH), $\nu_{max.}$ 3340br (OH), 1720 (C=O), 1605 and 1512 (arom.), 1282, 1120, and 1070 (C–O), and 710 cm^{-1} (C_6H_5), *m/e* 406 (M^+, 0·3%), 267, 249, 140, 123, 105 (100%), 77, and 43, δ (100 MHz; C_2D_6SO) 8·00 (2H, dd, *S* 8 and 2) and 7·8—7·4 (3H, m) (benzoate), 7·02 (1H, d, *S* 8), 6·76 (1H, d, *S* 2), and 6·49 (1H, dd, *S* 8 and 2) (phenolic aglycone), and 4·98 (1H, d, *S* 8, anomeric proton on C-1 of sugar). On keeping lacticolorin (210 mg) in pyridine (2 ml) and acetic anhydride (4 ml) for 16 h it afforded a product (373 mg) which was chromatographed to give *lacticolorin penta-acetate* (253 mg), m.p. 134—136° (from acetone with ether) (Found: C, 58·3; H, 5·1. $C_{30}H_{32}O_{14}$ requires C, 58·4; H, 5·2%), $\nu_{max.}$ 1755 and 1720 (C=O), 1605, 1515, 1370, 1280, 1230br, and 710 cm^{-1}, *m/e* 393, 231, 169, 109, 105 (100%), and 43, δ ($CDCl_3$) 8·3—6·9 (8H, m, arom.), 5·75 (1H, m, anomeric H), 5·0 (2H, s, benzylic H), 2·25, 2·16, and 2·06 (15H, 3s, acetate). Lacticolorin (1 g) in dimethylformamide (5 ml) was treated for 16 h with methyl iodide (12 ml) and silver oxide (3 g) according to Kuhn [35] and the product (1·2 g of a

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

light yellow syrup) was chromatographed to afford *lacticolorin penta-O-methyl ether* (435 mg), m.p. 102—105° (from light petroleum–ether) (Found: C, 62·8; H, 6·8. $C_{25}H_{32}O_9$ requires C, 63·0; H, 6·8%), $\nu_{max.}$ 1735 (C=O), 1600, 1520, 1280, 1100br, 805, and 705 cm^{-1}, *m/e* 476 (M^+, 4%), 309, 308, 277, 155, 105 (100%), 101, 88, 77, 75, 71, and 45, δ ($CDCl_3$) 8·0 (2H, dd, *S* 8 and 2·5) and 7·6—7·3 (3H, m) (benzoate), 7·0 (1H, d, *S* 8), 6·83 (1H, d, *S* 2), and 6·62 (1H, dd, *S* 8 and 2) (aglycone), 4·78 (1H, m, anomeric H), 4·32 (2H, s, benzylic H), and 3·80, 3·70, 3·65, 3·54, and 3·31 (5 × 3H, s, OCH_3).

Lacticolorin aryl methyl ether was obtained by treating lacticolorin (205 mg) in methanol (0·5 ml) and acetone (25 ml) with potassium carbonate (0·5 g) and methyl iodide (5 ml) under reflux until the solution showed no more free phenol with Pauly's reagent,[31] filtering, drying, and chromatographing the product to give crystals (94 mg), m.p. 134—137° (from water), R_F 0·44 in benzene–methanol–acetic acid (7 : 2 : 1 v/v),* $\nu_{max.}$ 3420br (OH), 2910 (CH), 1710 (C=O), 1598 and 1515 (arom.), 1470, 1454, 1320, 1285, 1228, 1160, 1135, 1070, 1030, and 711 cm^{-1} (monosubst. benzene ring), *m/e* 154 (100%), 137, 125, 123, 122, 105, 93, 91, 77, 73, 69, 65, 60, 57, 55, 51, 45, 43, and 41. Its *tetra-acetate* was obtained by keeping the compound (50 mg) in pyridine (1 ml) and acetic anhydride (2 ml) for 16 h and chromatographing the dried product; the chromatographically pure tetra-acetate (25 mg) did not crystallize and had R_F 0·44 in benzene–ethyl acetate (3 : 1 v/v), δ ($CDCl_3$) 5·76 (1H, m, anomeric H), 5·01 (2H, s, benzylic H in aglycone), 3·79 (3H, s, $ArOCH_3$), and 2·15, 2·08, 2·05, and 2·02 (4 × 3H, s, acetate).

Alkaline Hydrolysis of Lacticolorin.—Lacticolorin (340 mg) was refluxed with barium hydroxide octahydrate (500 mg) in water (40 ml) for 2 h. The solution was acidified (Congo Red) with N-sulphuric acid, the barium sulphate was filtered off, and the filtrate was extracted continuously with ether (8 h) to yield a residue (100 mg) which was sublimed and crystallized from light petroleum to give benzoic acid, m.p. and mixed m.p. 121·5—122·5° (Found: C, 68·9; H, 4·9. Calc. for $C_7H_6O_2$: C, 68·9; 4·95%); its i.r. spectrum was identical with that of pure benzoic acid. The aqueous solution was neutralized with barium carbonate, filtered, and dried to give *debenzoyl-lacticolorin* (245 mg) which could not be crystallized but showed only one spot at R_F 0·25 (*cf.* R_F 0·51 for lacticolorin on the same plate) in benzene–methanol–acetic acid (7 : 2 : 1 v/v). This material (83 mg) was kept in pyridine (1 ml) and acetic anhydride (3 ml) for 10 h and the dried product (157 mg) chromatographed to yield pure *debenzoyl-lacticolorin hexa-acetate* (X) (65 mg) as a glass which was distilled at 200—210° and 0·15 Torr (Found: C, 54·0; H, 5·4. $C_{25}H_{30}O_{14}$ requires C, 54·2; H, 5·45%), R_F 0·51 in benzene–ethyl acetate (3 : 1 v/v), $\nu_{max.}$ ($CHCl_3$) 3030, 2960, 2880, 1754 (C=O), 1511, 1370, 1225, and 1045 cm^{-1}, *m/e* 331, 169, 127, 109, and 43 (100%), δ (100 MHz; $CDCl_3$) 7·25—6·85 (3H, m, arom.), 5·68 (1H, m, anomeric H), 5·02 (2H, s, benzylic H), and 2·28, 2·16, 2·11, 2·09, and 2·05 (18H, 5s, acetate).

Debenzoyl-lacticolorin Aryl Methyl Ether Penta-acetate (XI).—The crude debenzoyl-lacticolorin (220 mg) in 50% aqueous acetone (20 ml) containing excess of solid potassium carbonate was treated under reflux with methyl iodide (10 ml) added in portions during 2 h. Insoluble material was filtered off and the filtrate was evaporated *in vacuo*. The product was dissolved in methanol (20 ml) and treated with methyl iodide (10 ml) and potassium carbonate for a further 3 h; the solution then gave no reaction with Pauly's reagent.[31] The product was chromatographed and dried (145 mg), kept in pyridine (5 ml) and acetic anhydride (10 ml) for 16 h, chromatographed again, and distilled at 230—240° and 0·2 Torr to give *debenzoyl-lacticolorin aryl methyl ether penta-acetate* (XI) as a glass which slowly crystallized, m.p. 88—93° (Found: C, 54·8; H, 5·9. $C_{24}H_{30}O_{13}$ requires C, 54·7; H, 5·7%), R_F 0·37 in benzene–ethyl acetate (3 : 1 v/v). $\nu_{max.}$ 2950, 2880, 1755 (C=O), 1599 and 1514 (arom.), 1378 1233, 1070, and 1047 cm^{-1}, *m/e* 526 (M^+, 0·2%), 331, 196, 169 (100%), 154, 137, 127, 109, 97, 81, and 43 (100%), δ ($CDCl_3$) 7·25—6·85 (3H, m, arom.), 5·75 (1H, m, anomeric H), 5·05 (2H, s, benzylic protons), 3·82 (3H, s, OCH_3), and 2·13, 2·10, 2·08, 2·06, and 2·02 (15H, 5s, acetate).

Debenzoyl-lacticolorin Hexa-O-methyl Ether (XII).—Debenzoyl-lacticolorin (208 mg) in dimethylformamide (5 ml) was treated with methyl iodide (5 ml) and silver oxide (1 g) as before.[35] The crude product (250 mg) was chromatographed and the pure ether (XII) (95 mg) obtained as a syrup showing a single spot at R_F 0·33 in benzene–ethyl acetate (7 : 3 v/v), *m/e* 386 (M^+, 3%), 219, 218 (100%), 187, 155, 127, 116, 111, 101, 99, 89, 88, 75, 73, 71, 59, 45, and 41, δ ($CDCl_3$) 7·1—6·6 (3H, m, arom.), 4·73 (1H, m, anomeric H), 4·34 (2H, s, benzylic H), and 3·80, 3·67, 3·62, 3·51, 3·35, and 3·33 (6 × 3H, s, OCH_3).

Acidic Hydrolysis of Lacticolorin.—Lacticolorin (56 mg) was refluxed in N-sulphuric acid (25 ml) for 6 h. The cooled solution was extracted continuously with ether (10 h), neutralized with solid barium carbonate, filtered, and evaporated *in vacuo* to give the crude sugar (22·5 mg), R_F 0·16 in benzene–methanol–acetic acid (7 : 2 : 1 v/v) (as also for authentic D-glucose), which (1 mg) was heated to *ca.* 65° for 2 min in pyridine (0·1 ml) containing *N*-trimethylsilylimidazole (Tri-sil 'Z') (0·15 mequiv.); on g.l.c. (6 ft column of 5% GE SE-52 on Chromosorb-W, 200°, flow rate 30 ml min^{-1}) this product showed two peaks at retention times of 10·3 and 14·4 min (as for α- and β-D-glucose under the same conditions), running concurrently with authentic D-glucose (anomeric mixture) silylated in the same manner.

Characterization of the Aglycone in Lacticolorin.—Debenzoyl-lacticolorin (85 mg) in 96% ethanol (25 ml) and 10M-hydrochloric acid (0·3 ml) was hydrogenolysed over 5% palladium–carbon (80 mg) at 1 Torr for 1 h. The solution was filtered; the filtrate was neutralized with Dowex 1-X8 resin (CO_3^{2-} form) and evaporated *in vacuo*. The residue, which showed only a single phenolic spot on t.l.c. [R_F 0·46 in benzene–methanol–acetic acid (7 : 2 : 1 v/v) as against R_F 0·24 for debenzoyl-lacticolorin on the same plate], was refluxed in N-sulphuric acid (30 ml) for 6 h and extracted continuously with ether for 24 h to yield a phenolic product [24 mg; R_F 0·25 in benzene–acetic acid (9 : 1 v/v) (as also for authentic 3,4-dihydroxytoluene on the same plate)], which was kept in methanol (1 ml) and 0·2M-diazomethane in ether (10 ml) for 16 h to afford 3,4-dimethoxytoluene (30 mg; b.p. 50—60° at 0·2 Torr), R_F 0·80 in benzene–ethyl acetate (9 : 1 v/v), identified by the identity of its i.r. and n.m.r. spectra with those of authentic 3,4-dimethoxytoluene. The point of attachment of the aglycone was determined by repeating the hydrogenolysis of debenzoyl-lacticolorin (300 mg) as before, keeping the

* Lacticolorin has the same R_F value in this system, but reacts positively with Pauly's reagent (orange spot).

first product (deoxydebenzoyl-lacticolorin) in methanol (1 ml) and 0·2M-diazomethane in ether (20 ml) for 16 h, and refluxing the crude product with N-sulphuric acid (30 ml) for 6 h. Continuous extraction (30 h) of the acidic solution with ether yielded 4-hydroxy-3-methoxytoluene (V) (65 mg), b.p. 60—70° at 0·04 Torr, identified by its i.r. and n.m.r. spectra, which were identical with those of authentic 4-hydroxy-3-methoxytoluene (also see later).

Degradation of Penta-O-methyl-lacticolorin (VI).—Penta-*O*-methyl-lacticolorin (VI) (384 mg) in methanol (10 ml) was refluxed for 3 h with barium hydroxide octahydrate (500 mg) in water (20 ml). Continuous extraction (48 h) with ether yielded the debenzoylated product [280 mg; crystalline, R_F 0·23 in benzene–ethyl acetate (2 : 1 v/v) against R_F 0·57 for the starting material]; continuous extraction (4 h) with ether after then acidifying the aqueous solution with hydrogen chloride gas afforded benzoic acid (98 mg), m.p. and mixed m.p. 121—121·5° (from water and from light petroleum) (Found: C, 68·7; H, 4·9. Calc. for $C_7H_6O_2$: C, 68·9; H, 4·95%), i.r. spectrum identical with that of pure benzoic acid. The debenzoylated product (280 mg) was hydrogenolysed over 10% palladium–carbon (150 mg) as before and the product [250 mg; R_F 0·32 in benzene–ethyl acetate (2 : 1 v/v)] was refluxed in methanol (10 ml) and N-sulphuric acid (40 ml) for 6 h. Ether extraction (45 min) of the cooled solution afforded 4-hydroxy-3-methoxytoluene (V) (79 mg), distilled at 55—60° and 0·1 Torr; this distillate (30 mg) in 10% sodium hydroxide solution (0·3 ml) was shaken with benzoyl chloride (40 mg) for 20 min to give 4-benzoyloxy-3-methoxytoluene, m.p. 72—73° (Found: C, 74·5; H, 5·85. $C_{15}H_{14}O_3$ requires C, 74·4; H, 5·8%), identical with an authentic preparation (mixed m.p. and i.r. spectrum). The acidic aqueous solution was neutralized with solid barium carbonate, filtered, and evaporated *in vacuo* at 40—45° to give a residue which on extraction with acetone gave a syrup (170 mg). This was chromatographed to afford pure 2,3,4-tri-*O*-methyl-D-glucose (82 mg) (Found for an undistilled[36] sample: C, 48·0; H, 8·3. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2%), $[\alpha]_D$ +68·5° (*c* 0·57). A reference sample of the same compound, prepared by acidic hydrolysis of tri-*O*-methyl-laevoglucosan,[36] had $[\alpha]_D$ +65·7° (*c* 0·80). The two samples furthermore had identical i.r. spectra[10] and showed identical behaviour of their trimethylsilyl ethers, both giving the same two (anomeric) peaks at retention times of 7·37 and 8·12 min on g.l.c. (carried out as before, at 180°), with no peak splitting on mixed injection with an authentic sample.

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[36] T. H. Evans, J. Levi, W. C. Hawkins, and H. Hibbert, *Canad. J. Res.*, 1942, **20***B*, 175.