Neriifolin, an Ester Glucoside of Benzene-1,2,4-triol

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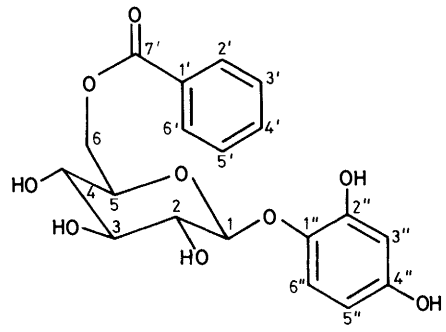
Neriifolin, a leaf metabolite of *Protea neriifolia* R. Br., is the 6-*O*-benzoyl-β-D-glucopyranoside of benzene-1,2,4-triol. The location of the benzoyloxy group on the sugar was directly confirmed by n.m.r. spectroscopy; the position of the glycosidic linkage to the benzenetriol was demonstrated by methylation and hydrolysis to 2,4-dimethoxyphenol (characterised by n.m.r. spectroscopy and by benzoylation).

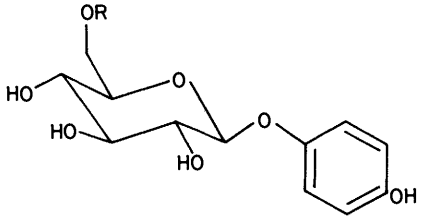
The major leaf metabolites of members of the genus *Protea* (family Proteaceae) so far studied [1–3] are aromatic esters of aryl glycosides. The units forming these structures comprise benzoate and cinnamate esters, D-glucose and D-allose as pyranoses, and hydroquinone and 3,4-dihydroxybenzyl alcohol as aglycones. A major leaf metabolite of *Protea neriifolia* R. Br., neriifolin, has now been shown to be a related ester glucoside where, however, the aglycone is benzene-1,2,4-triol. The latter compound has so far been encountered as a natural product only in lower plants, *viz.* a fungus [4] and a marine sponge.[5] Its occurrence in a higher plant is therefore notable.

Neriifolin (**1**), $C_{19}H_{20}O_9$, $[\alpha]_D$ −49° (*cf.*[1] eximin $[\alpha]_D$ −48°) was mostly obtained as a friable glass which could however also be obtained in crystalline form, m.p. 195 °C (see Experimental section). It was purified by repeated column chromatography to a single-spot material whose purity and homogeneity were assessed by silylation-g.l.c. (99% pure) and by its cleanly resolved spectra (see below). Its i.r. absorption was closely similar to that of the related ester glucoside, eximin (**2**).[1] Its composition was derived from the molecular ion found in the mass spectrum of its persilylated derivative.

Acidic hydrolysis of neriifolin (**1**) afforded the constituent parts of the molecule, *viz.* benzoic acid, D-glucose (characterised as its pentabenzoate), and benzene-1,2,4-triol. This triol was so reactive that it was completely degraded on contact with sodium hydrogen carbonate solution when its separation from benzoic acid was attempted. This did however allow the separation of a minor phenolic component, characterised as hydroquinone; a minor leaf metabolite accompanying neriifolin (see Experimental section) could therefore be of the eximin type. The mixture of benzoic acid, hydroquinone, and benzenetriol was therefore benzoylated and the identity of the recovered 1,2,4-tris(benzoyloxy)benzene confirmed by direct comparison with a synthetic sample.



(**1**)



(**2**) R = PhCO
(**3**) R = H

The n.m.r. characteristics of neriifolin differed from those of eximin in that the aglycone portion of the spectrum was that of a 1,2,4-trisubstituted benzene, but in other respects the spectra of the two compounds were closely similar (Tables 1—3). The ^{13}C resonances in Table 1 were assigned using C–H correlated spectra (XHCORR[6] and COLOC[7]). Because of the close coincidence of the resonance positions of 2-, 3-, and 4-H of both neriifolin and eximin, and of 2-, 3-, 4-, and 5-H of arbutin these techniques did not permit the assignment of signals due to the corresponding carbon atoms C-2, -3, and -4 (and also C-5 in the case of arbutin). The ^1H n.m.r. data in Tables 2 and 3 were obtained with the help of spin-echo correlated (SECSY[8]) spectra.

The point of attachment of the benzoyl group was indicated by the downfield shift by *ca.* 0.8 p.p.m. of the resonances of the geminal protons 6a and 6b, as in the case of eximin, relative[9] to the case of the debenzoyl analogue, arbutin (**3**). A separate n.m.r. experiment (COLOC)[7] directly confirmed the position of the ester linkage by demonstrating a ^3J spin coupling interaction between the benzoyl carbonyl carbon and the sugar proton 6a of neriifolin.

The β-configuration of the glycosidic linkage followed from the n.m.r. characteristics of 1-H (δ 4.55, $^3J_{1,2}$ 7.5 Hz); this proton is therefore[10] diaxial with respect to 2-H of the D-glucose portion of the molecule.

The position of attachment of the glycosidic link to the benzene-1,2,4-triol portion was acertained by methylation, under alkaline conditions, of the free phenolic groups of neriifolin, followed by acidic hydrolysis. The phenol so obtained was indicated to be 2,4-dimethoxyphenol by the melting point and the composition of its benzoate; no sample of authentic 2,4-dimethoxyphenol or its benzoate was however obtainable for purposes of direct comparison, and the disposition of the two methoxy groups relative to the phenolic hydroxy group was therefore independently established by an n.O.e. experiment on the free phenol. The coupling pattern observed for the resonances due to the aromatic protons of the free dimethoxyphenol is characteristic of a 1,2,4-trisubstituted benzene; irradiation of its undegassed solution at the frequency of the methoxy signal at δ 3.87 gave an n.O.e. at only 3″-H (10.5%) while irradiation at the frequency of the methoxy signal at δ 3.76 gave an n.O.e. at both 3″-H (4.0%) and at 5″-H (6.5%), thus confirming its substitution pattern.

Table 1. ^{13}C Chemical shifts (δ, at 50.32 MHz)

	1	2	3	4	5	6	1′	2′,6′	3′,5′	4′
Neriifolin[a]	105.73	(77.14	74.40	71.08)[b]	75.25	64.70	131.15	130.44	129.63	134.24
Eximin[c]	103.57	(77.95	74.93	72.06)	75.41	65.43	131.29	130.61	129.60	134.35
Arbutin[c]	103.57	(77.97	77.94	74.93	71.37)	62.50				

	7′	1″	2″	3″	4″	5″	6″
Neriifolin[a]	167.08	139.49	150.08	104.16	155.31	107.06	121.86
Eximin[c]	167.80	153.86	116.54	119.53	152.19	119.53	116.54
Arbutin[c]		152.39	116.59	119.34	153.74	119.34	116.59

[a] CD_3CN Solution. [b] Bracketed values are not firmly assigned. [c] CD_3OD solution. [d] The availability of a 200 MHz FT spectrometer facility allowed the definitive re-assignment of the 1, 5, 6a, and 6b proton resonances of eximin.[1]

Table 2. ^1H Chemical shifts (δ at 200.13 MHz)

	1	2	3	4	5	6a	6b	2′,6′	3′,5′	4′
Neriifolin[a]	4.55	~3.44	~3.44	~3.44	3.70	4.63	4.41	8.04	7.52	7.65
Eximin[a,d]	4.77	~3.47	~3.44	~3.44	3.73	4.63	4.41	8.03	7.53	7.66
Arbutin[a]	4.73	~3.35	~3.35	~3.35	~3.35	3.75	3.59			

	2″	3″	5″	6″	2-OH	3-OH	4-OH	6-OH	2″-OH	4″-OH
Neriifolin[a]		6.31	6.09	6.88	4.11	~3.65	~3.65		7.26	6.71
Eximin[a,d]	6.60	6.89	6.89	6.60	3.62	3.56	3.56			6.66
Arbutin[a]	6.71	6.91	6.91	6.71	~3.4	~3.4	~3.4	2.81		6.7 (br)

[a,b,c,d] See Table 1.

Table 3. Proton–proton coupling constants, J_{H-H} (Hz)

	1,2	4,5	5,6a	5,6b	6a,6b	3″,5″	5″,6″	6,6-OH
Neriifolin	7.5	9.2	2.3	6.4	12.0	2.8	8.6	
Eximin	7.5	9.4	2.3	6.9	11.9			
Arbutin	7.3		2.1	4.6	12.0			5.9

Experimental

Instruments used routinely were Kofler micro hot-stage (m.p.), Pye-Unicam SP3-300 (i.r. of potassium bromide dispersions), Bruker AC200 FT (n.m.r., 200 MHz), and Pye-Unicam GCD gas chromatograph (g.l.c.). T.l.c. was on silica gel plates (Merck F254), and column chromatography was over silica gel. Reversed phase paper chromatography, silylation-g.c., and the reading of emergent temperatures (ET) were carried out as described earlier.[11] Pauly's reagent is diazotised sulphanilic acid (50 mg) in aqueous sodium carbonate (1M; 10 ml).

Neriifolin.—Leaves of a cloned population of *Protea neriifolia* R. Br. originating from the Cape Outeniqua mountains and cultivated by the Protea Research Unit, Riviersonderend, were air-dried. A sample (0.2 g) was continuously extracted with methanol for 22 h to afford an extract which on t.l.c. (benzene–ethyl acetate–methanol, 4:1:1 v/v) with Pauly's reagent showed two major constituents as spots at hR_F* 30 (yellow) and 23 (dark slate). Bulk material was milled (110 g) and extracted continuously with methanol for 66 h, then solvent was evaporated to leave a dark tar (42 g). This was purified by repeated chromatography of the combined fractions (t.l.c.) in benzene–ethyl acetate–methanol mixtures to afford a glass (0.9 g) containing neriifolin as the major component by silylation–g.l.c (ET_{233} 272 °C, 91%), accompanied by a minor component (ET_{233} 267 °C, 8%. This enriched material served for chemical degradation (below).

A separate extraction of a further leaf sample (24 g) afforded similar results, and extended chromatography gave a purer product as a friable glass containing neriifolin (0.2 g, ET_{233} 272 °C, 99%) accompanied by less than 1% of two very minor contaminants with ET_{233} 268 °C and 276 °C. On t.l.c. and on reversed phase p.c.[11] it showed a single yellow spot with Pauly's reagent. Electron impact mass spectrometry of neriifolin although not showing a molecular ion, afforded a strong peak at m/z 105 for the benzoyl ion as the only significant result; the e.i. mass spectrum of the silylation product of neriifolin however showed a molecular ion at the value expected at $(M + 1)^+$ for $C_{34}H_{60}O_9Si_5$ and also the benzoyl ion: m/z 753 $(M + 1)^+$ (2), 482 (70), 271 (100), and 105 (30%); $[\alpha]_D$ −49° (*c* 0.38 in MeOH); $\nu_{max.}$ 3 400br (OH), 1 700 (PhC=O), 1 600, 1 510, and 1 450 (Ar), 1 280 and 1 070 (PhC–O), and 710 cm^{-1} (Ph); the differences between the absorptions of neriifolin and eximin are slight: the intensity of a peak at 970 cm^{-1} is markedly stronger in the former, while an absorption at 1 210 cm^{-1} (ArOH) in the latter is weaker and broader in the former. The n.m.r. characteristics are displayed in Tables 1—3.

A concentrated solution of glassy neriifolin in acetonitrile deposited microplatelets, m.p. 194.5—195 °C (from acetonitrile). The characteristics of crystallised neriifolin were identical with those of the purified glassy form as regards silylation–g.l.c., i.r., and ^1H and ^{13}C n.m.r. spectra (above).

Acidic Hydrolysis of Neriifolin.—(*a*) Enriched neriifolin (92% purity, 199 mg) in sulphuric acid (0.5M; 25 ml) was stirred under nitrogen at 95 °C for 5 h, and the cooled solution was extracted continuously with ether for 17 h. The aqueous layer was neutralised with an excess of barium carbonate and evaporated after clarification. The dried residue was leached with aqueous ethanol (50% v/v, 2 ml), which was then evaporated and dried to yield a clear gum (60 mg). Silylation-g.l.c. of this product showed that it was largely (90%) D-glucose, with two peaks for its silylated equilibrated anomers at ET_{122} 167 °C and 176 °C. This mixture (52 mg), dissolved in pyridine (2.5 ml) and benzoyl chloride (0.7 ml), was briefly heated and then kept at 25 °C for 18 h before being shaken with water (6 ml) for 4 h. Aqueous sodium hydrogen carbonate (1M; 11 ml) was added and the

* $hR_F = 100 \times R_F$.

mixture was extracted with chloroform to yield a gum (173 mg). Three crystallisations from ethyl acetate afforded pure β-D-glucose pentabenzoate (43 mg), m.p. 160—163 °C (lit.,[12] 156—158 °C), undepressed when mixed with an authentic sample similarly prepared from (+)-D-glucose. δ($CDCl_3$) Values were identical with those of an authentic preparation, consistent with reported values.[13]

The ether extract (above) was evaporated to afford a crystalline residue (132 mg). This mixture (128 mg) in ether (30 ml) was washed twice with aqueous sodium hydrogen carbonate (1M; 2 ml) which turned an intense red colour. Acidification and extraction with ether afforded benzoic acid (43 mg, silylation-g.l.c.: ET_{89} 107 °C, 100%) which was sublimed and crystallised from hexane and then hot water, m.p. (and mixed m.p.) 122—123 °C.

On silylation-g.l.c., the residue from the ether layer of the above alkaline extraction (24 mg) showed peaks for silylated hydroquinone (ET_{100} 121 °C) and benzene-1,2,4-triol (ET_{100} 139 °C), but during chromatography over silica gel the column turned black and the only product eluted was hydroquinone (8 mg), which (4 mg, ET_{100} 120 °C, 97%), on benzoylation in pyridine and benzoyl chloride as above, yielded hydroquinone dibenzoate (9 mg) which was chromatographed and crystallised to afford the pure compound, m.p. and mixed m.p. 203—204 °C; δ($CDCl_3$) 8.3—8.2 (4 H, m) and 7.7—7.5 (6 H, m) (benzoyl protons) and 7.29 (4 H, s, ArH).

(*b*) Enriched neriifolin (as above, 215 mg) in sulphuric acid (0.5M; 25 ml) was stirred under nitrogen at 95 °C for 2.5 h after which the solution was continuously extracted with ether for 18 h to yield a mixture (140 mg) of benzoic acid and phenols. This was chromatographed to afford a mixture (67 mg) which on silylation-g.l.c. showed peaks for benzoic acid (ET_{100} 114 °C, 31%), hydroquinone (ET_{100} 122 °C, 4%), and benzene-1,2,4-triol (ET_{100} 140 °C, 41%). This mixture (31 mg) was benzoylated in pyridine with benzoyl chloride as before and chromatographed to afford a mixture (41 mg) which on direct g.l.c. showed peaks for hydroquinone dibenzoate (ET_{245} 266 °C, 12%) and 1,2,4-tris(benzoyloxy)benzene (ET_{245} 313 °C, 88%). This difference in volatility made possible the removal of the less soluble and higher melting hydroquinone dibenzoate by subliming off about half the product up to 286 °C at 0.5 Torr; the remainder (21 mg) on g.l.c. showed only one peak (ET_{245} 313 °C, 100%). This was also sublimed (15 mg) and crystallised from ethyl acetate and ethanol (10 mg), m.p. 122 °C [when mixed with a reference sample of 1,2,4-tris(benzoyloxy)benzene, m.p. 123 °C (lit.,[14] 120 °C)]; δ($CDCl_3$) 8.3—8.0 (6 H, m) and 7.7—7.4 (9 H, m), 7.46 (1 H, d, *J* 9 Hz), 7.39 (1 H, d, *J* 3 Hz), and 7.26 (1 H, dd, *J* 9 and 3 Hz), Reference materials were prepared from benzene-1,2,4-triol[1] obtained *via* its triacetate.[16]

2,4-*Dimethoxyphenol from Neriifolin.*—Purified neriifolin (99%, 100 mg) was dissolved in water (0.13 ml) under nitrogen and two portions of sodium hydroxide solution (5M; 0.13 ml) and dimethyl sulphate (0.052 ml) were added over 5 min. The solution was stirred for 50 min, acidified to 0.5M with sulphuric acid (2 ml), kept under nitrogen at 100 °C for 4 h, and then extracted with ether to afford an oil (55 mg). Silylation-g.l.c. showed the presence of benzoic acid (ET_{100} 114 °C, 59%; t.l.c., hR_F = 35 in benzene–ethyl acetate–methanol, 8:1:1 v/v) and a phenol [ET_{100} 134 °C, 34%; t.l.c., (as above) hR_F = 69 and tan colouration with Pauly's reagent]. Chromatography afforded 2,4-dimethoxyphenol (17 mg, ET_{100} 134 °C, 99%); δ(CD_3CN) 6.72 (1 H, d, *J* 8.7 Hz), 6.54 (1 H, d, *J* 2.8 Hz), and 6.36 (1 H, dd, *J* 8.7 and 2.8 Hz), (ArH), 6.1 (1 H, s, OH), and 3.87 and 3.76 (2 × 3 H, s, OMe).

The phenol (9 mg) was further characterised as its benzoate [prepared using sodium hydroxide (2M) and benzoyl chloride], which after chromatography crystallised from aqueous ethanol as needles, m.p. 89 °C (lit.,[17] 90 °C) (Found: M^+, 258.0890. Calc. for $C_{15}H_{14}O_4$: *M*, 258.0891); δ($CDCl_3$) 8.2 (2 H, m) and 7.6—7.4 (3 H, m) (benzoate H), 7.06 (1 H, d, *J* 8.7 Hz), 6.59 (1 H, d, *J* 2.7 Hz), and 6.49 (1 H, dd, *J* 8.7 and 2.7 Hz) (ArH), and 3.83 and 3.80 (2 × 3 H, s, OMe). The structure of the phenol was confirmed by the n.m.r. experiment discussed earlier.

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References

1 G. W. Perold, M. E. K. Rosenberg, A. S. Howard, and P. A. Huddle, *J. Chem. Soc., Perkin Trans. 1*, 1979, 239.
2 G. W. Perold, P. Beylis, and A. S. Howard, *J. Chem. Soc., Perkin Trans. 1*, 1973, 638.
3 G. W. Perold, P. Beylis, and A. S. Howard, *J. Chem. Soc., Perkin Trans. 1*, 1973, 643.
4 R. von Ardenne and W. Steglich, *Z. Naturforsch. Teil. C*, 1974, **29**, 446.
5 S. J. Wratten and J. Meinwald, *Experientia*, 1981, **37**, 13.
6 E. Breitmaier and W. Voelter, 'Carbon-13 NMR Spectroscopy,' VCH, Weinheim, 1987, p. 92.
7 A. E. Derome, 'Modern NMR Techniques for Chemistry Research,' Pergamon, Oxford, 1987, p. 254.
8 K. Nagayama, A. Kumar, K. Wuthrich, and R. R. Ernst, *J. Magn. Reson.*, 1980, **40**, 321.
9 L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance in Organic Chemistry,' 2nd edn., Pergamon, Oxford, 1969, p. 164.
10 J. M. van der Veen, *J. Org. Chem.*, 1963, **28**, 564; B. Capon and D. Thacker, *Proc. Chem. Soc.*, 1964, 369.
11 G. W. Perold, L. Carlton, A. S. Howard, and J. P. Michael, *J. Chem. Soc., Perkin Trans. 1*, 1988, 881.
12 J. J. Willard, J. S. Brimacombe, and R. P. Brueton, *Can. J. Chem.*, 1964, **42**, 2560.
13 N. B. D'Accorso, I. M. E. Thiel, and M. Schueller, *Carbohydr. Res.*, 1983, **124**, 177.
14 J. Thiele and K. Jaeger, *Ber. Dsch. Chem. Ges.*, 1901, **34**, 2837.
15 M. Healey and R. Robinson, *J. Chem. Soc.*, 1934, 1625.
16 E. B. Vliet, 'Organic Syntheses,' 2nd edn., Wiley, 1944, Coll. vol. 1, p. 317.
17 E. Spaeth, M. Pailer, and G. Gergely, *Ber. Dsch. Chem. Ges.*, 1940, **73**, 795, 802.

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