POLYPRENOLS FROM THE LEAVES OF *QUERCUS ILEX* INFECTED BY *MICROSPHAERA ALPHITOIDES*

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ABSTRACT.—C_{45}, C_{50}, C_{55} and C_{60} polyprenols with usual isoprenoid unsaturation were isolated from *Q. ilex* leaves infected by fungus *M. alphitoides*. The most abundant C_{55} prenol was found to be identical with already known ficaprenol-11. The assignment of all the signals in its ^{13}C-nmr spectrum confirmed the alignment of the *Z* and *E* internal residues. A structure-based naming system for unsaturated polyprenols was suggested.

In connection with our interest in the chemical alteration produced by biotic injuries on vegetal tissues, we have extensively studied the terpenoids of galls produced by insects on several species of the genera *Pistacia* and *Quercus* (1,2).

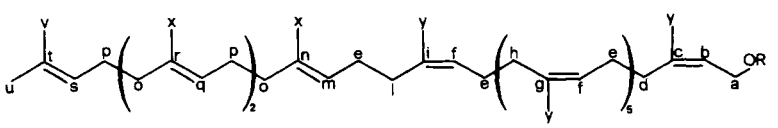
In a novel approach to the same problem, we now report a chemical investigation on the leaves of *Q. ilex*, a fagaceous species indigenous to southern Europe and northern Africa infected by *Microsphaera alphitoides* (3), a pathogenic fungus of *Oidium* genus. This microorganism is known to attack all the species of *Quercus* and other plants such as *Castanea sativa* and *Fagus sylvatica* and is, therefore, the cause of considerable ecological and economic damages.

DISCUSSION

The leaves collected from a specimen infected by *M. alphitoides* were extracted with hexane and then with ether. The ethereal extract was found to contain a fraction (40–250 mg per kg of fresh leaves) that could not be detected in the leaves of an uninfected plant; this fraction was separated by Silica gel column chromatography.

Such a fraction consisted of a mixture of polyprenols and was resolved into four pure compounds through reversed phase preparative tlc (4).

Spectra (ms, ir and nmr) indicated their identity with C_{45}, C_{50}, C_{55} and C_{60} polyprenols, consisting of three internal *E* and four, five, six and seven internal *Z* isoprene residues, respectively, besides the ω and the α *Z* terminal units. The same polyprenols have been found in *Ficus elastica* (5) and in *Cleome spinosa* (6) and are very probably identical with those from *Betula verrucosa* (7), *Aesculus hippocastanum* (8), *Hevea brasiliensis* (9) and *Aglaonema robelinii* (9).



1 a R= H b R= COCH$_3$

As far as the most abundant C_{55} alcohol **1a** is concerned, the ^{1}H-nmr spectrum and the ^{13}C-nmr spectrum were quite similar to those reported for ficaprenol-11 by Stone *et al.* (5) and Tanaka and Takagi (10).

By comparison with the data reported for isomeric 3,7,11-trimethyl dodeca-2,6,10-trienes (11) and 1,4-*cis*- and 1,4-*trans*-polyisoprenes (12), we have been able to assign all the signals in the ^{13}C-nmr spectrum of **1a**. This result allowed

us to confirm the alignment $\omega,E,E,E,Z,Z,Z,Z,Z,Z,\alpha$ OH attributed to ficaprenol-11 by the Japanese authors, and, on the other hand, to amend some assignments proposed by these authors.

In our opinion, the signal at δ 29.64 attributed to an impurity is due instead to the C–1 methylene of the *Z* terminal unit. Its multiplicity (triplet in off resonance spectrum) strongly supports this view even if the 2.6 ppm upfield shift relative to the corresponding methylene of an internal *Z* unit could not be confidently anticipated.[1] In addition the intensity ratio (2.97 : 4.85 : 1.05 : 1) of signals at δ 39.73 (*trans, trans*-CH_2), δ 32.21 (*cis,cis*-CH_2), δ 31.99 (*trans,cis*-CH_2) and δ 29.64 –*cis*,terminal *cis*-CH_2) agrees much better with the structure **1a** than the 2.8 : 6.3 : 0.9 ratio of signals due to *trans,trans*-CH_2, *cis,cis*-CH_2 and *trans,cis*-CH_2, as reported by the Japanese authors. Furthermore the ^{13}C-nmr spectrum of acetate **1b** was identical with that of the parent alcohol, the signals relative to the terminal unit excepted. Significantly the signal at δ 29.64 assigned to C_1 methylene was shifted downfield to δ 30.30.[2]

As far as C–4 methylenes are concerned, the signals at δ 26.64 and 26.37 were assigned to *trans*-CH_2, *trans* and *cis*-CH_2, *cis* respectively. The intensity ratio 3 : 7.1 of these signals suggests however that *trans*-CH_2, *cis* overlaps *cis*-CH_2, *cis*. The upfield shift of 0.27 ppm of a C_4 in the *trans* unit, *cis* linked, relative to a *trans* linked one was verified also in *Z,E*- and *E,E*-3,7,11-trimethyldodeca-2,6,10-trienes (11).

Comparison with the pertinent data from the above-mentioned trienes led to assignment of all vinylic carbons excepting those present in the α unit, which were recognized through the shifts produced by acetylation.

TABLE 1. ^{13}C-nmr chemical shifts of [3*E*, 7*Z*]–11–prenol (**1a**).

a	b	c	d	e	f	g	h
59.02 (*t*) (61.09)	125.12 (*d*) (119.23)	139.70 (*s*) (142.61)	29.64 (*t*) (30.30)	26.37 (*t*)	125.06 (*d*)	135.42 (*s*)	32.21 (*t*)
i	l	m	n	o	p	q	r
134.85 (*s*)	31.99 (*t*)	124.19 (*d*)	136.18 (*s*)	39.73 (*t*)	26.64 (*t*)	124.30 (*d*)	135.25 (*s*)
s	t	u	v	x	y		
124.57 (*d*)	131.28 (*s*)	25.61 (*q*)	17.65 (*q*)	15.97 (*q*)	23.38 (*q*)		

In bracket are reported the shieldings of acetate **1b**, when different.

C_{55} prenol from *Q. ilex*, therefore, has the structure **1a**, the same assigned to ficaprenol-11 and cleomeprenol-11.

In this regard, it seems the trivial names derived from natural sources should be dropped. Since the IUPAC nomenclature for these compounds is rather cumbersome,[3] we suggest a simple structure-based naming system.

It is suggested that polyprenols be named merely as prenols prefixed by the number of prenyl units contained. Thus **1a** is an 11-prenol. Further, it is suggested that this name be prefixed by stereochemical designators indicating from the ω end the number of contiguous *Z* units, then the number of contiguous *E* units, then the number of *Z* units, and so on until the α unit has been included. Thus **1a** is [3*E*,7*Z*]-11-prenol.

[1]Unfortunately a possible comparison (the chemical shift of methyls at C–3 and C–7 in farnesol) is meaningless owing to the disagreement between available data (13).

[2]A comparable shift downfield has been reported for the vinylic methyl in acetylgeraniol (14).

[3]The IUPAC name of the compound **1a** is 3,7,11,15,19,23,27,31,35,39,43-undecamethyl-2*Z*, 6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*E*,34*E*,38*E*,42-tetratetracontaundecaen-1-ol.

Finally, as the biological role of fully unsaturated polyprenols in the higher plants is an open question (15), their presence in the leaves of *Q. ilex* only when infected by *M. alphitoides* might be of some relevance.

EXPERIMENTAL[4]

ISOLATION AND PURIFICATION OF POLYPRENOLS.—*Q. ilex* and *M. alphitoides* were identified by Prof. G. Aliotta of the University of Naples. The leaves (500 g), collected from an infected specimen growing in the Botanical Garden of the University, were extracted with hexane and then with ether. The ethereal extract (7 g) was directly chromatographed on a Si gel column. A fraction (185 mg) eluted with benzene, when rechromatographed [Si gel; petroleum ether-diethyl ether (19:1)], gave a mixture of polyprenols (60 mg). The polyprenol content increased from March to July changing from 40 to 250 mg per kg of fresh leaves. The mixture of polyprenols was subjected to reversed phase preparative tlc (50 mg each plate; acetone-water (9:1); fluorescein). Each band was extracted with ether separately to give polyprenol-9, -10, -11 and -12.

HPLC ANALYSIS OF *p*-NITROBENZOYL DERIVATIVES OF POLYPRENOLS.—Aliquots of single polyprenols (5 mg) were treated with *p*-nitrobenzoyl chloride (0.05 ml) in dry pyridine (0.5 ml). After 3 hrs, ether was added and the ethereal layer was washed with 0.2 *N* HCl and water until neutral. After removal of the solvent, the residue was dissolved with propan-1-ol (0.5 ml). Hplc analysis showed a purity better than 95% for every compound.

The percentage of each polyprenol in the mixture was determined in the same way and was found to be: C_{45} (3.1%), C_{50} (22.0%), C_{55} (61.3%), C_{60} (13.6%). An aliquot (100 mg) of ethereal extracts from infected and uninfected leaves was treated with *p*-nitrobenzoyl chloride in pyridine. Comparative Hplc analyses showed peaks attributable to polyprenols only in infected samples.

9-PRENOL.—Spectral data obtained for 9-prenol was as follows: ir: νmax (CCl_4) 3570, 3305, 1005 (OH), 1660, 840 (isolated C=C) cm^{-1}; ms: *m/z* 630 (3%), 612 (5), 543 (5), 475 (7), 407 (6), 339 (7), 271 (9), 203 (16), 135 (26), 121 (40), 95 (65), 81 (74), 69 (100); ^{1}H-nmr: δ 1.62 (*s*, 12 H, *E* CH_3-C=C), 1.65 (*s*, 15 H, *Z* CH_3-C=C), 1.75 (*s*, 3 H, *Z* CH_3-C=C-CH_2OH), 2.02 and 2.05 (*ss*, 32 H, allylic CH_2), 4.09 (*d*, 2 H, *J*=6.7 Hz, -CH_2OH), 5.09 (*m*, 8 H, vinylic CH), 5.43 (*t*, 1 H, *J*=6.7 Hz, *CH*-CH_2OH).

10-PRENOL.—Spectral data obtained for 10-prenol was as follows: ir: νmax (CCl_4) 3565, 3300, 1660, 1000 and 840 cm^{-1}; ms: *m/z* 698 (4%), 680 (12), 543 (3), 475 (4), 407 (4), 339 (6), 271 (10), 203 (13), 135 (37), 121 (51), 95 (75), 81 (90), 69 (100); ^{1}H-nmr: δ 1.62 (*s*, 12 H), 1.65 (*s*, 18 H), 1.75 (*s*, 3 H), 2.02 and 2.04 (*ss*, 36 H), 4.09 (*d*, 2 H), 5.09 (*m*, 9 H), 5.43 (*t*, 1 H).

[3*E*, 7*Z*]-11-PRENOL (**1a**).—Compound **1a** gave the following spectral data: ir: νmax (CCl_4) 3575, 3305, 1660, 1005 and 843 cm^{-1}; ms: *m/z* 766 (4%), 748 (10), 679 (4), 611 (4), 543 (3), 475 (5), 407 (5), 339 (4), 271 (5), 203 (10), 135 (21), 121 (35), 95 (50), 81 (70), 69 (100); ^{1}H-nmr: δ 1.62 (*s*, 12 H), 1.65 (*s*, 21 H), 1.75 (*s*, 3 H), 2.02 and 2.05 (*ss*, 40 H), 4.08 (*d*, 2 H), 5.09 (*m*, 10 H), 5.42 (*t*, 1 H).

ACETYLATION OF [3*E*, 7*Z*]-11-PRENOL (**1a**).—Compound **1a** (40 mg) was treated with acetic anhydride (0.5 ml) in dry pyridine (1 ml) overnight. The mixture was added to a mixture of ether and water. The ethereal layer, when washed with *N* HCl and then water until neutral and evaporated *in vacuo*, gave acetate **1b**. The purity of this compound was found to be better than 95% by glc (Perkin-Elmer 3920B chromatograph (FID) equipped with a 1.83 x 2 mm i.d. glass column containing 2.5% OV-1 on Chromosorb G AW-DMCS). It gave the following spectral data: ir: νmax CCl_4) 1736, 1240, 1660, 835 cm^{-1}; ^{1}H-nmr: δ 2.05 (*s*, 3 H, CH_3OCO), 4.57 (*d*, 2 H, CH_2OAc), 5.37 (*t*, 1 H, =*CH*-CH_2OAc).

12-PRENOL.—Spectral data for 12-prenol was as follows: ir: νmax (CCl_4) 3565, 3305, 1660, 1005 and 840 cm^{-1}; ms: *m/z* 834 (3%), 816 (9), 747 (5), 679 (5), 611 (4), 543 (3), 475 (6), 407 (5), 339 (5), 271 (4), 203 (10), 135 (22), 121 (38), 95 (45), 81 (73), 69 (100); ^{1}H-nmr: δ 1.62 (*s*, 12 H), 1.65 (*s*, 24 H), 1.75 (*s*, 3 H), 2.02 and 2.05 (*ss*, 44 H), 4.09 (*d*, 2 H), 5.09 (*m*, 11 H), 5.43 (*t*, 1 H).

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[4] ^{1}H- (270 MHz) and ^{13}C-nmr (67.88 MHz) spectra were performed with the assistance of I. Giudicianni at the Centro di Metodologie Chimicofisiche of the University on a Bruker WH 270 FT spectrometer with ASPECT 2000 computer in $CDCl_3$ solns with TMS as internal standard. Proton noise decoupled and off resonance decoupled ^{13}C spectra yielded chemical shifts and differentiated carbon types as reported for **1a** and **1b**. The quantitative measurements were obtained by inverse-gated decoupling to eliminate NOE effects with a microprogram identical with that one described in the Bruker ASPECT 2000 NMR software manual (pulse interval 15 sec, pulse width 5 μsec, decoupling power 5 watt): such conditions ensure significant data (16). Mass spectra were determined with an MS 9 (AEI) spectrometer. Hplc analysis was carried out on a Varian model 5000 apparatus equipped with an uv detector (254 nm) using a CH-10 Micropack reverse phase column (30 cm x 4 mm i.d.) and propan-1-ol as eluent (17). Reversed phase analytical and preparative tlc were performed according to Dunphy *et al.* (4).

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