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ISOPRENOIDS FROM THE LEAVES OF *QUERCUS SUBER*

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ABSTRACT.—Besides seventeen already known lupane and oleanane triterpenes, epibetulinic aldehyde has been isolated from the leaves of *Quercus suber*. In addition, 9-, 10-, 11-, and 12-prenol were present in specimens infected by the fungus *Microsphaera alphitoides*.

In connection with the chemical investigation of alterations produced by biotic injuries in the tissues of plants of the *Quercus* genus, we recently reported (1) a study of the leaves of *Quercus ilex* L. infected by the fungus *Microsphaera alphitoides*. In this paper, we report a chemical investigation of the leaves of *Quercus suber* L. infected by the same fungus.

Because no phytochemical data concerning the leaves of *Q. suber* were available, we previously studied the tissues from uninfected specimens to compare them with infected ones. The leaves collected from plants growing in the botanical garden of the University of Naples were air dried and treated with cold Et_2O to afford a crude extract that was separated into acidic and neutral fractions by conventional procedures.

The acidic fraction, after repeated chromatography on HCl-washed silica gel, gave six triterpene acids with the lupane and oleanane skeletons. They were purified and identified as the corresponding methyl esters and are described in Table 1 (2-5).

TABLE 1. Methyl Esters of Acidic Triterpenes from *Quercus suber* Leaves

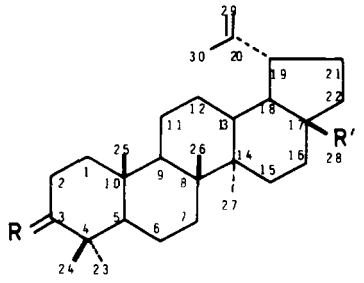
Methyl esters		mp	$[\alpha]_D$	%Amount[a]	Reference
Betulinate	**1h**	221-223°	+ 5°	16.6	(2)
3-O-Acetyl betulinate	**1i**	201-204°	+19°	9.0	(2)
Betulonate	**1l**	164-165°	+31°	5.0	(3)
3 Epibetulinate	**1m**	221-222°	−13°	3.6	(4)
Oleanolate	**2h**	195-197°	+82°	15.6	(5)
Oleanonate	**2l**	181-182°	+76°	6.0	(5)

[a]The % amount refers to the acidic fraction.

The neutral fraction was chromatographed on neutral alumina; subsequent chromatography afforded twelve triterpenes listed in Table 2 (6-11). Eleven were already known and were identified on the basis of their physical characteristics and by comparison with authentic samples.

Compound **1g**, mp 185-187° $[\alpha]_D$+4° was characterized as 3α-hydroxy-lup-20(29)-en-28-al (3-epibetulinic aldehyde); its ir spectrum showed absorptions at 3620 (OH), 2705, and 1735 (CHO) cm^{-1}; the pmr spectrum closely resembled that of betulinic aldehyde **1e** showing the C-25, C-26, C-27, and C-30 methyl signals at δ 0.82, 0.92, 0.98, and 1.68, respectively, the H-19 signal at δ 2.99, and the formyl proton at δ 9.66. The replacement of the 3β-hydroxyl group with 3α- one was effective on the shieldings of the C-23 and C-24 methyl groups and the 3β geminal proton which were shifted to δ 0.93, 0.83 and 3.39, respectively. Jones oxidation and the subsequent esterification with ethereal CH_2N_2 of **1g** gave methyl betulonate **1l**.

In Table 3, cmr shieldings of epibetulinic aldehyde are reported, as well as those of other lupane compounds variously oxidized at C-3 and C-28 so as to complete the data already reported by Wenkert *et al.* (12) and Sholichin *et al.* (13). The signal assignments were carried out through single frequency off-resonance decoupling experiments and by



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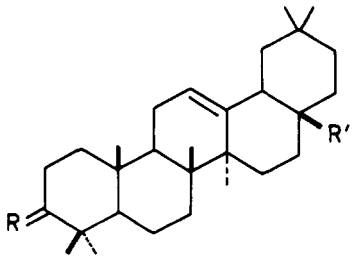

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a R=H,βOH R′=CH_3
b R=O R′=CH_3
c R=H,βOH R′=CH_2OH
d R=H,βOAc R′=CH_2OAc
e R=H,βOH R′=CHO
f R=O R′=CHO
g R=H,αOH R′=CHO
h R=H,βOH R′=$COOCH_3$
i R=H,βOAc R′=$COOCH_3$
l R=O R′=$COOCH_3$
m R=H,αOH R′=$COOCH_3$

TABLE 2. Neutral Triterpenes from *Quercus suber* Leaves

Compounds		mp	[α]D	% Amount[a]	Reference
Lupeol	**1a**	212-214°	+ 27°	16.6	(6)
Lupenone	**1b**	168-170°	+ 61°	3.0	(6)
Betuline	**1c**	254-256°	+ 19°	9.3	(7)
3,28-O-Diacetyl betuline	**1d**	214-215°	+ 21°	3.3	(7)
Betulinic aldehyde	**1e**	192-193°	+ 19°	1.5	(8)
Betulonic aldehyde	**1f**	165-166°	+ 52°	1.3	(8)
3 Epibetulinic aldehyde	**1g**	185-187°	+ 4°	1.0	—
β-Amyrine	**2a**	198-199°	+ 88°	7.2	(9)
β-Amyrenone	**2b**	176-178°	+107°	4.5	(9)
Erythrodiol	**2c**	231-234°	+ 79°	11.8	(10)
Oleanolic aldehyde	**2e**	169-172°	+ 72°	2.4	(10)
Oleanolic aldehyde	**2f**	138-139°	+ 88°	1.7	(11)

[a]The % amount refers to the neutral fraction.

comparison with the reported data (14) for other pentacyclic triterpenes. Comparison of the spectra of **1d-1m**, in the light of substitution induced shifts, was also useful for determination of the shift allocations. The replacement of the 3β-hydroxyl group with 3α- one results in significant upfield shifts of C-2, C-3, and C-4; the C-23 methyl resonance is practically unchanged, whereas the C-24 signal shifts about 7 ppm downfield. Substitution of the C-28 methyl with differently oxidized groups significantly affects C-17 (downfield shift) and C-16 and C-22 (comparable upfield shifts).

The leaves collected from infected specimens afforded the same pattern of triterpene compounds in addition to a mixture of polyprenols that was resolved into four pure compounds through reversed-phase preparative tlc (1). Spectral analysis (ms, ir, and nmr) indicated their identity with 9-, 10-, 11-, and 12-prenol 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. Their *p*-nitrobenzoyl derivatives were compared by hplc with authentic samples showing that such a mixture was identical with that already isolated from the leaves of *Quercus ilex* infected by the same fungus (1).

TABLE 3. Cmr Data of Lupane Triterpenes

Atoms	1d	1e	1g	1i	1l	1m
C-1	38.4	38.7	33.6	38.2	39.6	33.6
C-2	23.6	27.3	25.9[a]	23.7	34.1	25.9[a]
C-3	80.8	78.9	76.4	81.0	217.9	76.3
C-4	37.7	38.8	37.5	37.8	47.3	37.6
C-5	55.4	55.5	49.9[b]	55.4	55.0	50.0[b]
C-6	18.1	18.2	18.4	18.1	19.6	18.5
C-7	34.1[a]	34.3	34.4	34.2	33.6	34.6
C-8	40.9	40.8	41.0	40.6	40.6	41.3
C-9	50.3	50.4	50.5[b]	50.3	49.9	50.7[b]
C-10	37.0	37.1	37.3	37.0	36.9	37.7
C-11	20.9	20.7	20.8	20.8	21.4	21.0
C-12	25.2	25.5	25.6[a]	25.4	25.5	25.7[a]
C-13	37.6	38.7	38.7	38.2	38.2	38.6
C-14	42.7	42.5	42.6	42.3	42.4	42.8
C-15	27.0	29.2[a]	29.5	30.5	29.6	29.9
C-16	29.8[b]	28.8[a]	28.8	32.1	32.1	32.4
C-17	46.3	59.3	59.3	56.5	56.5	56.9
C-18	47.7	48.0[b]	48.0[c]	46.9	46.9	47.3
C-19	48.8	47.5[b]	47.5[c]	49.4	49.4	49.4
C-20	150.0	149.7	149.8	150.5	150.4	150.7
C-21	29.6[b]	29.8	30.0	30.5	30.6	31.0
C-22	34.5[a]	33.2	33.2	37.0	36.8	37.1
C-23	27.9	27.9	28.2	27.9	26.6	28.2
C-24	16.4[c]	15.4	22.2	16.5[a]	21.0	22.2
C-25	16.1[c]	15.9[c]	15.9[d]	16.4[a]	15.7[a]	16.2[c]
C-26	16.0[c]	16.1[c]	16.1[d]	16.0[a]	15.9[a]	16.0[c]
C-27	14.7	14.2	14.2	14.6	14.6	14.9
C-28	62.7	205.6	205.6	176.6	176.5	176.6
C-29	109.8	110.1	110.1	109.6	109.6	109.5
C-30	19.1	19.0	19.0	19.3	19.3	19.5

[a,b,c,d]Assignments bearing the same superscript in anyone spectrum may be reversed.

Knowledge concerning the biological role of polyprenols is still limited; however, α-saturated polyprenyl phosphates seem to be the lipids involved in the glycosylation of glycoproteins in higher plants (15), whereas no lipid intermediate is involved in the synthesis of polysaccharides in fungi (16), with the exception of an aquatic fungus (17). Thus, the presence of 9-, 10-, 11-, and 12-α unsaturated prenols in the leaves of *Q. ilex* and *Q. suber*, only when infected by *M. alphitoides*, might be helpful in the understanding of their biochemical roles.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Pmr (270 MHz) and cmr (67.88 MHz) spectra were performed 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. Mass spectra were determined with a Kratos MS 50 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 × 4 mm i.d.) and *n*-PrOH as eluent.

PLANT MATERIALS.—The leaves of *Q. suber* were collected from the botanical garden, Naples, Italy. *Q. suber* and *M. alphitoides* were identified by G. Aliotta, professor of Botany, University of Naples.

EXTRACTION AND ISOLATION OF ISOPRENOIDS.—Dried leaves (700 g) of uninfected specimens were extracted with cold Et_2O. The ethereal extract (9 g) was treated with activated charcoal to eliminate most of the chlorophylls and was subsequently separated into an acidic (500 mg) and a neutral fraction (1400 mg) by conventional procedures.

SEPARATION OF THE ACID COMPONENTS.—The acid extract was absorbed on HCl-washed silica gel (30 g): elution with petrol-Et_2O (9:1, 300 ml) gave a less polar fraction (120 mg); further elution with petrol-Et_2O (7:3, 600 ml) afforded a second fraction (200 mg). The first fraction was treated with ethereal CH_2N_2 (excess), and the crude product (125 mg) was chromatographed on silica gel (5 g). Elution with petrol gave a mixture of two substances subsequently separated by preparative tlc and identified as ketoesters **1l** (25 mg) and **2l** (30 mg). Elution with petrol-Et_2 (9:1) gave acetoxyester **1i** (45 mg). The second fraction was esterified with $CH_2N_2$and chromatographed on silica gel (6 g) to afford methyl epi-betulinate **1m** (18 mg; petrol-Et_2O, 4:1): pmr δ 0.82 (s, 3H), 0.83 (s, 3H), 0.91 (s, 3H), 0.92 (s, 3H), 0.97 (s, 3H), 1.67 (s, 3H), 2.97 (m, 1H), 3.39 (t, 1H), 3.66 (s, 3H), 4.60, and 4.73 (ss, 2H); methyl betulinate **1h** (83 mg; petrol-Et_2O, 7:3) and methyl oleanolate **2h** (78 mg; petrol-Et_2O, 7:3).

SEPARATION OF THE NEUTRAL COMPONENTS.—The neutral extract was absorbed on neutral alumina (45 g). Petrol eluted lupenone **1b** (42 mg) and β-amyrenone **2b** (63 mg). Elution with petrol-Et_2O (19:1) gave ketoaldehydes **1f** (19 mg) and **2f** (24 mg) and diacetate **1d** (46 mg). Elution with petrol-Et_2O (9:1) gave lupeol **1a** (230 mg) and β-amyrine **2a** (101 mg). Further elution with the same solvent gave epibetulinic aldehyde **1g** (14 mg), which after crystallization from hexane-C_6H_6 (7:3) had mp 185-187°; $[\alpha]_D$ +4° (c 1.0); ms *m/z* 440; pmr δ 0.82 (s, 3H), 0.83 (s, 3H) 0.92 (s, 3H), 0.93 (s, 3H), 0.98 (s, 3H), 1.68 (s, 3H), 2.99 (m, 1H), 3.39 (t, 1H), 4.60, and 4.73 (ss, 2H), 9.66 (s, 1H). Jones oxidation and subsequent esterification of **1g** (10 mg) gave ketoester **1l** (mp and mmp 164-166°). Petrol-Et_2O, 4:1) eluted betulinic **1e** (21 mg) and oleanolic **2e** (33 mg) aldehydes. Finally, elution with petrol-Et_2O (7:3) gave diols **1c** (130 mg) and **2c** (166 mg).

IDENTIFICATION OF POLYPRENOLS IN THE LEAVES OF INFECTED SPECIMENS.—The leaves (750 g) collected from an infected specimen were treated as above. Elution with petrol-Et_2O (19:1) of the neutral fraction gave a mixture (155 mg) of triterpenes and polyprenols, which was treated with *p*-nitrobenzoyl chloride (excess) in dry pyridine. The crude reaction mixture was chromatographed on alumina (5 g): petrol eluted the mixture of polyprenyl *p*-nitrobenzoates, which was checked by hplc analysis and found to consist of 9-, 10-, 11- and 12-prenyl derivatives in a 0.1:0.3:1.0:0.5 ratio. $LiAlH_4$ reduction of the mixture of prenyl *p*-nitrobenzoates gave four free alcohols that were separated through reversed phase preparative tlc (1) and found identical with pure samples of 9-, 10-, 11-, and 12-prenol.

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