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SECOIRIDOIDS FROM *OLEA EUROPAEA**

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Key Word Index—*Olea europaea*; Oleaceae; secoiridoids; ligstroside; ^{1}H and ^{13}C NMR.

Abstract—Three new secoiridoids have been isolated from the leaves of *Olea europaea* along with the previously reported. The compounds were identified by spectral means.

INTRODUCTION

Olea europaea L. contains the secoiridoid glucoside, oleuropein (**1**) [1–3] and its demethyl derivative, demethyloleuropein (**1a**) [4].

Both substances stimulate oviposition of *Dacus oleae* (Gmel.), an insect which infests Mediterranean olive crops [4, 5]. The water hydrolysate of oleuropein, mainly constituted of the aglycone, shows the same activity, whilst chloroform extracts of the hydrolysate are more active [6, 7]. It is noteworthy that degradation products from the hydrolysis of oleuropein inhibit oviposition of *D. oleae* and this fact is attributable to the formation of 3,4-dihydroxy-β-phenylethanol [6, 7].

As a part of our studies of the natural substances active on the environmental behaviour of *D. oleae*, we have re-examined the constituents of the extracts from olive leaves, and have isolated the new secoiridoids **2** and **3**, the oleoside **10** and ligstroside (**9**), a constituent frequently isolated from other oleacea species [8, 9].

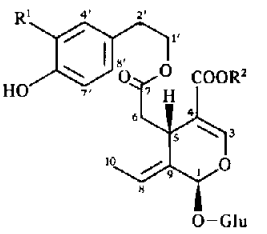
RESULTS AND DISCUSSION

The methanol extractable material of olive leaves was taken up with water and acetone, and successively extracted with *n*-pentane, chloroform and ethyl acetate. Column chromatography of the chloroform fraction gave a mixture of two products (**2** and **3**) in the ratio 1:7 (HPLC and ^{1}H NMR), which were separated from each other by medium pressure column chromatography (checking the purity of the fractions by HPLC analysis). Compounds **2** and **3** were isolated as viscous liquids which gave a positive ferric chloride test. Several derivatives of **2** and **3**, i.e. 2,4-dinitrophenylhydrazones, condensation products with dimedone, thiosemicarbazones, which were easily obtained under standard conditions, were also non-crystalline.

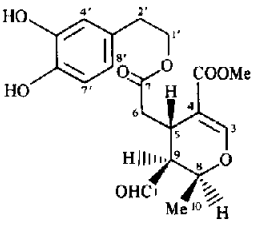
The IR spectra ($CHCl_3$) of compounds **2** and **3** were consistent with the presence in both compounds of an aldehydic group (2830, 1730 cm^{-1}), carbonyl groups and double bonds (1670–1720 cm^{-1}), and an aromatic ring (1630 cm^{-1}). Their UV spectra [λ_{max}^{MeOH} nm (log ε): 280 (3.5) and 230 nm (4.1)] were identical as were their EI-mass spectra [*m/z* 378 $[M]^+$ (5%), 346 $[M-MeOH]^+$ (6%) and 136 (base peak)].

Both compounds contained a 3,4-dihydroxyphenylethanol fragment and a carbomethoxy group on an enol ether double bond as in the case of oleuropein (**1**), and, as shown by the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), one methyl group on an oxygen-bearing secondary carbon.

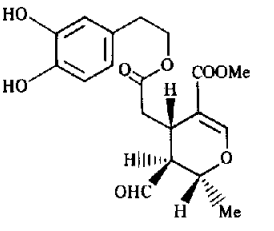
Spin decoupling experiments established the carbon sequence of compound **2**. Thus on irradiation at δ9.61, the signal at δ2.62 was mainly affected, while irradiation at δ4.18 modified the signals at δ1.56 and δ2.62, indicating the fragment Me–CH(O)–CH–CHO. Coupling between the



	R^1	R^2
1	OH	Me
1a	OH	H
9	H	Me



2*



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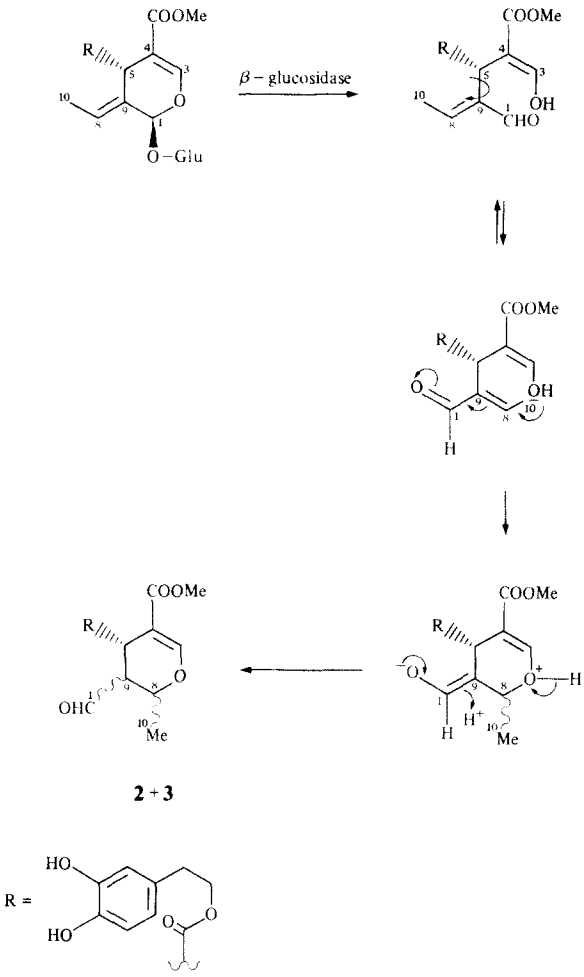
* Numbering based on possible biogenetic origin from oleuropein (see Scheme 1)

* Part of this work has been presented at the II Meeting on *Dacus oleae*, Perugia, March 1982.



Scheme 1.

signals at δ3.37 and those at δ2.22 and δ2.86, together with coupling between the signals at δ3.37 and at δ2.62 confirmed the sequence C-6, C-5, C-9. A 0.9 Hz allylic coupling between H-5 and the vinyl proton at δ7.66 confirmed the presence of a dihydropirane ring with a secoiridoid-type carbon atom sequence. Coupling between the 2H multiplet at δ2.82 and the two protons at δ4.20 and δ4.37 along with the aromatic proton pattern allowed identification of the 3,4-dihydroxyphenylethanol moiety, which is also found in oleuropein (**1**) (see Table 1).

The ^{1}H NMR data of compound **2**, excluding the phenylethanol signals, were in good agreement with that described for elenolic acid (**4**), a secoiridoid formerly isolated by Panizzi *et al.* [1] from among the acid catalysed hydrolysis compounds of oleuropein and whose structure and absolute stereochemistry was elucidated by MacKellar *et al.* [10], who also accomplished the total synthesis of (*R,S*)-methyl elenolate [11]. Some values of coupling constants reported in ref. [10] differed from ours, namely $J_{5,9}$ which was given the value of 2.5 Hz for elenolic acid (1.3 Hz in compound **2**) and $J_{8,9}$ = 8.5 Hz described in the same paper for methyl elenolate (2.5 Hz in compound **2**).

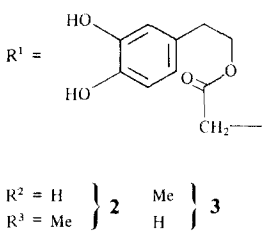
Later on R. T. Brown *et al.* [12] performed the transformation of secologanin into elenolic acid (**4**) and its methyl ester (**5**), again describing characteristics for both compounds virtually identical to those of ref. [10]. $J_{5,9}$ was not measured, whereas a value of 2.5 Hz was assigned to $J_{8,9}$ which is consistent with our findings for compound **2**.

Any attempt to transform compound **2** into elenolic acid (**4**) or into its methyl ester (**5**) (acid or base catalysed hydrolysis, transesterification with MeOH in the presence of Et_3N or of Ph_3P and CH_2=CH–CN [13]) failed; in all cases the yields were very low and a mixture of compounds was obtained. With the purpose of trying the opposite transformation, we synthesized elenolic acid (**4**) by acid catalysed hydrolysis of oleuropein. Again this compound, as well as its methyl ester, displayed, after purification, a rather high sensitivity to temperature and chemical treatments. Both **4** and **5** when submitted to accurate ^{1}H NMR analysis at 200 MHz gave very similar results to compound **2**. As shown in Table 1, all the chemical shifts and the coupling constants have very similar values. In particular, $J_{5,9}$ had a value of 1.3 Hz in **2** and 1.4 Hz in **4** and **5**; furthermore, two peculiar long-range couplings ($^4J_{1,8}$ and $^5J_{3,9}$) were identical both in **2** and **5**. CD measurements on **2** and **4** showed again close similarity; both compounds displayed only one negative Cotton effect at 222 nm with similar ellipticity ($-260\,000$ and $-200\,000$ respectively).*

All these data strongly support the structural and stereochemical identity of the secoiridoid moiety of the two substances **2** and **4**. If this is so then the values of $J_{5,9}$ for compound **4** and $J_{8,9}$ for compound **5** reported in ref. [10] are incorrect and must be assigned the ones shown in Table 1.

Formulae **6** and **7** represent the two half-chair conformations for the dihydropirane ring of these com-

*In ref. [12] a value of $[\theta]_{223} = -530\,000$ is reported for impure (~ 80%) compound **4**.



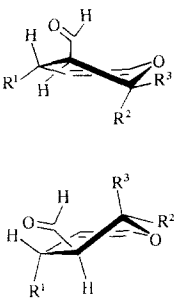


Table 1. ^{1}H NMR spectral data of compounds 1–5 [200 MHz, DMSO-d_6 (1) or $CDCl_3$, TMS as int. standard]

H	1	2	3	4	5
1	5.80 (*br s*, $J_{1,10}$ = 1.5 $J_{1,8}$ = 1.5)	9.61 (*dd*, $J_{1,9}$ = 2.1, $J_{1,8}$ = 1.0)	9.50 (*d*, $J_{1,9}$ = 1.8)	9.63 (*br s*, $J_{1,9}$ = 2.1)	9.63 (*dd*, $J_{1,9}$ = 2.1, $J_{1,8}$ = 1.0)
3	7.46 (*s*)	7.66 (*br s*, $J_{3,5}$ = 0.9, $J_{3,9}$ = 0.5)	7.57 (*d*, $J_{3,5}$ = 1.4)	7.65 (*br s*, $J_{3,5}$ = 1.0)	7.63 (*br s*, $J_{3,5}$ = 1.0, $J_{3,9} \simeq 0.5$)
5	3.86 (*dd*, $J_{5,6b}$ = 9.0, $J_{5,6a}$ = 3.0)	3.37 (*m*, $J_{5,9}$ = 1.3, $J_{5,6b}$ = 2.6, $J_{5,6a}$ = 11.2)	3.38 (*m*, $J_{5,9}$ = 5.5, $J_{5,8}$ = 1.1, $J_{5,6b}$ = 3.5, $J_{5,6a}$ = 8.6)	3.38 (*m*, $J_{5,9}$ = 1.4, $J_{5,6a}$ = 3.0, $J_{5,6b}$ = 11.0)	3.38 (*m*, $J_{5,9}$ = 1.4, $J_{5,6a}$ = 3.2, $J_{5,6b}$ = 11.1)
6a	2.62 (*dd*, $J_{6a,6b}$ = 14.0)	2.86 (*dd*, $J_{6a,6b}$ = 16.0)	2.88 (*dd*, $J_{6a,6b}$ = 16.2)	3.01 (*dd*, $J_{6a,6b}$ = 16.6)	2.93 (*dd*, $J_{6a,6b}$ = 16.1)
6b	2.40 (*dd*)	2.22 (*dd*)	2.53 (*dd*)	2.32 (*dd*)	2.12 (*dd*)
8	5.92 (*dq*, $J_{8,10}$ = 8.0)	4.18 (*m*, $J_{8,10}$ = 6.7, $J_{8,9}$ = 2.5)	4.46 (*ddq*, $J_{8,10}$ = 6.6, $J_{8,9}$ = 5.5)	4.22 (*br q*, $J_{8,10}$ = 6.8, $J_{8,9}$ = 2.4)	4.20 (*bq*, $J_{8,10}$ = 6.7, $J_{8,9}$ = 2.5)
9	—	2.62 (*m*)	2.57 (*dt*)	2.66 (*m*)	2.64 (*m*)
10	1.64 (*dd*)	1.56 (*d*)	1.38 (*d*)	1.58 (*d*)	1.58 (*d*)
COOMe	3.64 (*s*)	3.74 (*s*)	3.72 (*s*)	3.75 (*s*)	3.74 and 3.70 (*s*)
1'a	4.06 (*m*)	4.37 (*m*)	4.28 (*dt*, $J_{1'a,1'b}$ = 11.0, $J_{1'a,2'}$ = 6.6)	—	—
1'b		4.20 (*m*)	4.26 (*dt*, $J_{1'b,2'}$ = 6.6)	—	—
2'	2.63 (*br t*, main J = 7.0)	2.82 (*m*)	2.80 (*br t*)	—	—
4'	6.60 (*d*, $J_{4',8'}$ = 2.0)	6.85 (*d*, $J_{4',8'}$ = 2.0)	6.78 (*d*, $J_{4',8'}$ = 2.0)	—	—
7'	6.64 (*d*, $J_{7',8'}$ = 8.5)	6.81 (*d*, $J_{7',8'}$ = 8.0)	6.74 (*d*, $J_{7',8'}$ = 8.0)	—	—
8'	6.64 (*dd*)	6.63 (*dd*)	6.60 (*dd*)	—	—
Glucose					
1	4.66 (*d*, J = 8.0)	—	—	—	—

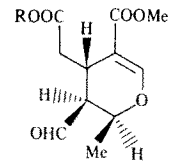
Phenolic protons are at δ5.55 (1H, *br s*) and δ6.85 (1H, *br s*) and disappear on D_2O treatment.

Table 2. ^{13}C NMR chemical shifts of compounds 1–3

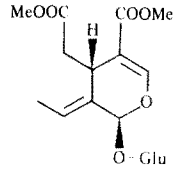
C	1 (DMSO-d_6)	2 ($CDCl_3$)	3 ($CDCl_3$)
1	93.96*	199.55	199.62
3	154.10*	156.90	153.38
4	129.85	130.09	129.88
5	30.88	28.08	26.79
6	40.43*	38.88	36.89
7	171.51	170.92	171.45
8	123.90*	69.57	70.53
9	129.49	50.89	54.12
10	13.55	17.80	19.06
COOMe	167.02	167.11	167.14
COOMe	51.88	51.63	51.28
1′	65.76	65.36	64.96
2′	34.37*	34.20	33.95
3′	108.46	106.34	106.10
4′	116.25	115.08	114.95
5′	145.53	143.29	143.23
6′	144.17	142.85	142.52
7′	116.82	116.21	115.78
8′	120.41	120.97	120.75
Glucose			
1	99.79	—	—
2	73.87	—	—
3	77.08	—	—
4	70.61	—	—
5	77.69	—	—
6	61.77	—	—

Assignments were made by consideration of chemical shifts and residual splittings in the off-resonance spectra. Carbons 4 and 9 were assigned by observing the different long-range splittings in the ^{1}H-coupled spectrum.

*Assigned by selective ^{1}H decouplings.



4 R = H
5 R = Me



10

pounds. Taking into account the low value of $J_{5,9}$ in **2**, **4** and **5** (1.3, 1.4 Hz) an average conformation near to **6** should be preferred by them in solution. Thus the *trans* pseudoequatorial–equatorial relationship between H-5 and H-9 can lead to a torsion angle $\phi_{5,9} = 100°$ and to $\phi_{9,8} = 55°$ which is in agreement with the observed coupling constant $J_{9,8} = 2.4$, 2.5 Hz.

^{1}H NMR analysis together with spin decoupling experiments were run on compound **3** as described for **2** and led to the conclusion that the two substances were diastereoisomers. A comparison between the ^{1}H NMR data of **2** and **3** (Table 1) revealed the following: (a) the C-10 methyl group resonated at lower field ($\Delta\delta = 0.18$ ppm) in **2**; (b) the opposite phenomenon was displayed by H-8 ($\Delta\delta = 0.28$ ppm); (c) H-5 had a similar chemical shift in both compounds ($\Delta\delta = 0.01$ ppm).

From (a) and (b), it can be concluded that H-8 must be *cis* with respect to the aldehyde group in compound **3** whereas it is *trans* in compound **2**. Whatever the conformation of the dihydropirane ring, the anisotropy induced by the carbomethoxy group on H-5 (pseudo-axial or pseudo-equatorial) should be very similar. Therefore (point c), H-5 must be oriented toward the aldehyde group both in **2** and in **3**. The average conformation in solution for compound **3** should be near to **7** with the two bulky groups (CHO and Me) rather equatorial and a sensible ring flattening (the oxygen atom moving slightly up) which allows the attainment of the torsion angles between H-5, H-9 and H-9, H-8 of ~ 130° corresponding to the observed coupling constants ($J_{5,9} = J_{9,8} = 5.5$ Hz).

2D-NOE (NOESY) experiments were run to confirm the stereochemistry of **3** and the conformation of both **2** and **3**. A pulse sequence utilizing regular increments of the 'mixing time' was used to minimize coherences arising from scalar couplings [14]. Several starting values of the mixing time (0.1 up to 0.5 sec) were tried, but in no case were any useful results obtained; only obvious NOE effects were detected between the geminal protons H-6a and H-6b, H-1a and H-1b (only in compound **2**) and between the vicinal protons H-1′ and H-2′, H-7′ and H-8′.

Since the absolute configuration of oleuropein was given [2, 3], and accepting for **2** and **3** a derivation from oleuropein as shown in the formulae, it is possible to assign the stereochemistry 5*S*,9*S*,8*S* to compound **2** and to propose a 5*S*,9*S*,8*R* configuration for compound **3**.

Column chromatography of the crude acetate extract allowed the isolation of oleuropein (**1**), 3,4-dihydroxy-β-phenylethanol (**8**), ligstroside (**9**) and small amounts of oleoside (**10**). The structure of the latter compound was determined by comparing its spectroscopic properties with those of oleuropein (**1**).

As **10** is a new compound in Nature, its structure and stereochemistry were confirmed by acetylation which gave a crystalline compound whose melting point and $[\alpha]_D^{25}$ were in agreement with those reported by Inouye *et al.* for a compound obtained after saponification, esterification and acetylation of oleuropein [2, 3].

Compound **8** was previously isolated from vegetation waters of olive fruits [15, 16]. Compound **9** has been obtained from different oleacea species eg Fraxinus, Ligustrum and Syringa [8, 9; Teresawa, M., private communication].

EXPERIMENTAL

^{1}H and ^{13}C NMR: 200 and 50.31 MHz respectively, TMS as internal standard. 16 K data point used for ^{1}H NMR acquisition (4 sec); time exponential and Gaussian apodization functions

applied to FID's to optimize resolution (RE = 0.1, AF = 0.4). TLC: silica gel GF_{254} (Merck), spots visualized either under UV light (254 nm) or by spraying with H_2SO_4 followed by heating; HPLC: Hibar Lichrosorb Si60 (Merck) 10 μm column (25 × 0.4 cm stainless steel), $CHCl_3$–*n*-hexane–MeOH–HCO_2H (60:40:2:1), flow rate 1.5 ml/min, pressure 33 kg/cm^2, UV absorption at 254 nm; EIMS: 70 eV. Plant material was collected by one of us (L. V.) in a private estate on Pistoia hills (Italy) in January 1982 of the quality Leccino, Moraiolo, Frantoio.

Isolation of **2** *and* **3**. Fresh leaves (2.0 kg) were macerated in 5 l. of MeOH for a week, at room temp. The solvent was evaporated under vacuum, and the residue taken up with 500 ml of Me_2CO–H_2O (1:1). The aq. mixture was successively extracted with *n*-pentane, $CHCl_3$ and EtOAc. After solvent evaporation, the extracts weighed 36.0 g (*n*-pentane), 27.1 g ($CHCl_3$) and 11.2 g (EtOAc). The viscous $CHCl_3$ extract (1.5 g) was submitted to CC using 50 g silica gel 60 (Merck, 70–230 mesh) and eluting with 400 ml $CHCl_3$ followed by 500 ml $CHCl_3$–MeOH (49:1). Combined fractions gave 402 mg of **2** and **3** which was re-chromatographed on a pre-packed Lobar Si 60 column (Merck, 24 cm × 1 cm i.d.) eluting with $CHCl_3$–*n*-hexane–MeOH–HCO_2H (55:45:1.5:1, 2 kg/cm^2), to give 30 mg **2** and 345 mg **3**. Secoiridoid **2** showed: $[\alpha]_D^{25}$ −44.0° (*c* 0.72; $CHCl_3$); $[\theta]_{222}$ = −260000 (MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2830, 1730, 1720–1670, 1630; UV λ_{max}^{MeOH} nm (log ε): 280 (3.5), 230 (4.1); MS *m/z* (rel. int.): 378 $[M]^+$ (5), 346 (6), 243 (3), 225 (6), 211 (6), 196 (5), 136 (100). Secoiridoid **3** showed the same IR, UV and mass spectra as **2**; $[\alpha]_D^{25}$ +35.7° (*c* 0.70; $CHCl_3$); $[\theta]_{242}$ = +42000, $[\theta]_{218}$ = −140000 (MeOH).

Acid hydrolysis of oleuropein (**1**) was carried out as described in ref. [1]. Oleuropein (507 mg) was treated with 1 M H_2SO_4 (5 ml); dioxane (1 ml) was added to dissolve the sample and the mixture left at room temp. for 3 days. After this period, the dioxane was evaporated under vacuum, $NaHCO_3$ was added and the soln was extracted with EtOAc. The aq. phase was treated with 1 M HCl, then extracted with $CHCl_3$ (5 × 5 ml). After drying and solvent evaporation, 90 mg of crude elenolic acid were obtained. The crude acid was chromatographed on silica gel (10 g) eluting with $CHCl_3$–*iso*-PrOH (49:1) + 1% HCOOH (TLC were run in the same eluent × 3), and the acid-enriched fractions (48 mg) purified on silica gel (10 g) eluting with $CHCl_3$–*iso*-PrOH (99:1) + 1% HCOOH, to give 16 mg elenolic acid (**4**). Oil, UV λ_{max}^{MeOH} nm (log ε): 237 (3.94); $[\theta]_{222}$ = −200000 (MeOH); ^{1}H NMR see Table 1.

On treatment of **4** with ethereal CH_2N_2, the methyl ester **5** was obtained. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1710, 1630; UV λ_{max}^{MeOH} nm: 234; ^{1}H NMR: see Table 1.

Isolation of oleuropein (**1**), *ligstroside* (**9**), *oleoside* (**10**) *and* 3,4-*dihydroxy-β-phenylethanol* (**8**) *from the acetate extract*. The crude acetate extract (6.86 g) was separated by CC using 200 g silica gel and eluting with 1 l. of $CHCl_3$–MeOH (9:1), then with 400 ml $CHCl_3$–MeOH (4:1). Combined fractions gave 5.9 g oleuropein (**1**): spectral data identical to those reported in the lit. [2, 3]. The first fractions of this chromatography (720 mg) were rechromatographed using 50 g silica gel and eluting with 900 ml $CHCl_3$–MeOH (99:1), then with 400 ml of $CHCl_3$–MeOH (9:1). Combined fractions gave 147 mg 3,4-dihydroxy-β-phenylethanol (**8**) as the less polar compound, 50 mg oleoside (**10**), and 120 mg ligstroside (**9**). Compounds **9** and **8** showed the same spectral data as reported in the literature. Compound **10** showed $[\alpha]_D^{25}$ −121.0 (*c* 1; MeOH); IR ν_{max}^{nujol} cm^{-1}: 3400, 1724, 1705, 1635; UV λ_{max}^{EtOH} nm (log ε): 233 (3.89); ^{1}H NMR (DMSO-d_6): δ 7.24 (H-3, *s*), 5.82 [H-8, *q* (*br*) 8], 5.54 [H − 1, *s* (*br*)], 3.48 (COOMe, *s*), 3.38 (COOMe, *s*), 2.28 (H-6a, *dd*, 14, 4), 2.12 (H-6b, *dd*, 14, 10), 1.26 [H-10, *d* (*br*), 8]; MS *m/z* (rel. int.): 238 (39), 224 (29), 196 (35), 178 (100), 165 (98), 151 (31).

Acetylation of oleoside (**10**). Compound **10** (10 mg) was treated with Ac_2O (0.5 ml) and C_5H_5N (0.5 ml) overnight at room temp. The reaction mixture was poured into ice-water and extracted several times with $CHCl_3$. The crude product was purified by prep. TLC (0.25 mm, double developed with petrol–EtOAc, 1:1) and crystallized from EtOAc–petrol (4:1), mp 117–118° (lit. 114.5–116° [2, 3]); $[\alpha]_D^{25}$ −162° (*c* 0.45; $CHCl_3$) (lit. −163.4° [2, 3]).

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