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β-AMYRIN ACETATE EPOXIDE FROM *CANARINA CANARIENSIS*

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Key Word Index—*Canarina canariensis*; Campanulaceae; leaf wax; β-Amyrin acetate epoxide; 3β-acetoxyoleanan-12-one; taraxerone; isomultiflorenol acetate; 2D NMR.

Abstract—Among the triterpenoids isolated from the leaf wax of *Canarina canariensis*, a new oleanane derivative, 3β-acetoxy-12α,13α-epoxyoleanane, has been identified. When deuterochloroform is used as NMR solvent, the compound isomerizes to 3β-acetoxyoleanan-12-one. The structural formulae of these compounds were established from one- and two-dimensional NMR data.

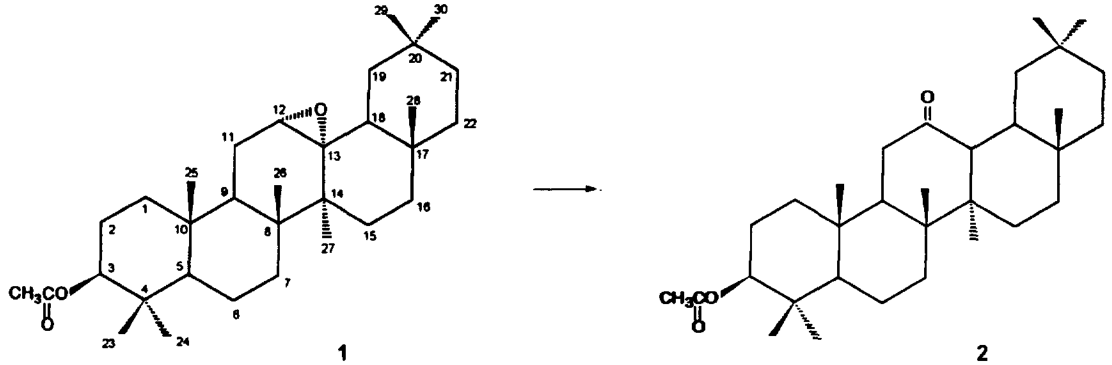
INTRODUCTION

Canarina canariensis is a plant endemic to the Canary Islands Tenerife, Gran Canaria, La Palma and Gomera that produces beautiful bell-shaped red flowers. It occurs in laurel forests and with *Erica arborea* shrubs between 100 and 300 m above sea level. Its leaves and stems exhibit a thin waxy coating. We have now analysed this lipophilic material and have identified its major components.

RESULTS AND DISCUSSION

From the leaf and stem wax, a new oleanane derivative, 3β-acetoxy-12α-13α-epoxyoleanane **1** (Fig. 1) was isolated and identified. This compound has now been characterized for the first time as a natural product but was previously synthesized from β-amyrin acetate using *p*-nitroperbenzoic acid by Boar *et al.* [1]. Moreover, compound **1** rearranged in $CDCl_3$ to give 3β-acetoxyoleanan-12-one **2** (Fig. 1). Therefore, to avoid a mixture which precludes the use of a two-dimensional NMR approach, **1** was studied in C_6D_6 solution. Finally, it should be noted that similar isomerization has also been observed in the case of sclareol epoxide [2].

The ^{13}C NMR spectrum of **1** consists of 32 resolved signals. Beyond confirming the presence of an acetate function, the multiplicities of the individual signals determined using the DEPT pulse sequence [3] indicated a trisubstituted epoxide function, six quaternary carbons, eight methyl groups, and three methine and eleven methylene resonances. Close inspection of the 400 MHz ^1H NMR spectrum {C_6D_6; δ 4.66 (1H, *dd*,



1 **2**

Fig. 1. β-Amyrin acetate epoxide **1** and its $CDCl_3$ isomerization product 3β-acetoxyoleanan-12-one **2**.

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$J = 12.0$, 4.7 Hz, $C\underline{H}O$), 2.59 (1H, *dbr*, $J = 4.0$ Hz, $C\underline{H}O$), 1.74 (3H, *s*, $C\underline{H}_3CO$), 1.29 (6H, *s*), 1.10 (3H, *s*), 0.94 (3H, *s*), 0.88 (3H, *s*), 0.87 (3H, *s*), 0.79 (3H, *s*), 0.78 (3H, *s*)} showed a hydrogen α to an acetate function, a tertiary epoxide ring and eight methyls linked to aliphatic quaternary carbons. The presence of two *gem*-dimethyl groups was further supported by the number of sp^3-hybridized quaternary carbons. From the above results, a proposed molecular formula was established as $C_{32}H_{52}O_3$, which was verified by the $[M]^+$ in the mass spectrum at m/z 484. The structure of β-amyrin acetate epoxide **1** and, therefore, its ^1H and ^{13}C NMR spectral parameters, were deduced from the concerted application of homonuclear and both direct and long-range heteronuclear chemical shift correlation techniques. One-bond proton–carbon chemical shift correlations were established using a ^1H-detected one-bond (C, H) heteronuclear multiple quantum coherence (HMQC) experiment [4], providing the identities of the direct responses as shown in Table 1.

When dissolved in deuterochloroform, **1** isomerizes slowly to give a new product **2**. Its ^{13}C NMR spectrum showed the presence of a carbonyl group at δ 215.1 and an acetate function at δ 171.0. Examination of the 400 MHz ^1H NMR data was indicative of eight quaternary methyl groups, a methine proton linked to an oxygen-bearing carbon and a resonance of a deshielded methylene adjacent to a ketone function {$CDCl_3$; δ 4.49 (1H, *dd*, $J = 10.5$, 5.5 Hz, $C\underline{H}O$, 2.29 (1H, *dd*, $J = 16.1$, 6.0 Hz, $C\underline{H}_2CO$), 2.10 (1H, *dd*, $J = 16.1$, 12.3 Hz, $C\underline{H}_2CO$), 2.03 (3H, *s*, $C\underline{H}_3CO$), 1.18 (3H, *s*), 0.97 (3H, *s*), 0.96 (3*H*, *s*), 0.84 (3H, *s*)}. The molecular framework, and the complete ^1H and ^{13}C chemical shift assignments (Table 2) of **2**, were deduced as for **1**, on the basis of concerted application of two-dimensional experiments.

Among the other compounds characterized, taraxerone, first isolated from the bark of *Pieris japonica* [5], was identified on the basis of the general similarity of its ^1H and ^{13}C NMR chemical shifts with those of previously reported data [6]. Another triterpene derivative, isomultiflorenol acetate was also identified. This

Table 1. ^1H and ^{13}C NMR chemical shifts of β-amyrin acetate epoxide (**1**)

$CDCl_3$			C_6D_6			
Atoms	$\delta\,^{13}C$*	Group†	Atoms	$\delta\,^{13}C$*	Group†	$\delta\,^1H$*
C-1	37.82	CH_2	C-1	37.81	CH_2	1.23 and 0.71
C-2	23.58	CH_2	C-2	23.87	CH_2	1.69 and 1.53
C-3	80.92	CH	C-3	80.50	CH	4.66
C-4	37.82	C	C-4	37.90	C	—
C-5	55.61	CH	C-5	55.81	CH	0.63
C-6	17.75	CH_2	C-6	17.97	CH_2	1.35
C-7	34.19	CH_2	C-7	34.45	CH_2	1.26 and 1.08
C-8	40.44	C	C-8	40.61	C	—
C-9	46.51	CH	C-9	46.72	CH	1.22
C-10	37.25	C	C-10	37.33	C	—
C-11	21.73	CH_2	C-11	22.02	CH_2	1.79 and 1.45
C-12	54.80	CH	C-12	54.49	CH	2.59
C-13	67.46	C	C-13	66.87	C	—
C-14	41.31	C	C-14	41.55	C	—
C-15	24.50	CH_2	C-15	24.87	CH_2	2.05 and 0.99
C-16	27.93	CH_2	C-16	28.28	CH_2	1.97 and 0.97
C-17	33.54	C	C-17	33.78	C	—
C-18	48.41	CH	C-18	48.62	CH	1.10
C-19	41.31	CH_2	C-19	41.65	CH_2	1.73 and 1.13
C-20	30.93	C	C-20	30.94	C	—
C-21	34.72	CH_2	C-21	34.96	CH_2	1.33 and 1.12
C-22	37.10	CH_2	C-22	37.48	CH_2	1.49 and 1.22
C-23	16.57	CH_3	C-23	16.79	CH_3	0.88
C-24	28.06	CH_3	C-24	28.11	CH_3	0.87
C-25	15.20	CH_3	C-25	15.26	CH_3	0.78
C-26	20.42	CH_3	C-26	20.70	CH_3	1.29
C-27	22.08	CH_3	C-27	22.20	CH_3	1.10
C-28	28.47	CH_3	C-28	28.85	CH_3	1.29
C-29	33.37	CH_3	C-29	33.44	CH_3	0.94
C-30	23.53	CH_3	C-30	23.55	CH_3	0.79
CO	171.10	C	CO	169.96	C	—
$COCH_3$	21.39	CH_3	$COCH_3$	20.82	CH_3	1.74

*In ppm with respect to TMS.

†Determined from DEPT spectra.

Table 2. ^{1}H and ^{13}C NMR chemical shifts of 3β-acetoxyoleanan-12-one (**2**)

Atoms	$\delta\,^{13}$C*	Group†	$\delta\,^1$H*
C-1	38.68	CH_2	1.60 and 1.10
C-2	23.58	CH_2	1.66 and 1.61
C-3	80.61	CH	4.49
C-4	37.82	C	—
C-5	55.57	CH	0.85
C-6	18.08	CH_2	1.54 and 1.37
C-7	34.76	CH_2	1.23 and 1.18
C-8	43.79‡	C	—
C-9	49.11	CH	1.74
C-10	37.48	C	—
C-11	38.50	CH_2	2.29 and 2.10
C-12	215.10	C	—
C-13	59.96	CH	1.90
C-14	41.96‡	C	—
C-15	27.02	CH_2	1.84 and 1.01
C-16	30.31	CH_2	1.62 and 0.93
C-17	31.66	C	—
C-18	34.33	CH	2.30
C-19	43.62	CH_2	1.45 and 1.04
C-20	30.91	C	—
C-21	34.92	CH_2	1.66 and 1.40
C-22	35.99	CH_2	1.35
C-23	16.97	CH_3	0.87
C-24	28.25	CH_3	0.85
C-25	16.30	CH_3	0.96
C-26	19.34	CH_3	0.97
C-27	28.25	CH_3	1.18
C-28	28.25	CH_3	0.89
C-29	31.91§	CH_3	0.90
C-30	27.87§	CH_3	0.845
CO	171.03	C	—
$COCH_3$	21.36	CH_3	2.03

*In ppm with respect to TMS.

†Determined from DEPT spectra.

‡§These assignments may be reversed.

compound was first isolated from the wax gourd *Benincasa cerifera* [7] and our NMR results are in agreement with previously published values [8].

EXPERIMENTAL

General. All 1D and 2D NMR spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ or C_6D_6 solns (^{1}H at 400 MHz; ^{13}C at 100.61 MHz; TMS as standard in both measurements). Standard Bruker pulse sequences were used for homonuclear and heteronuclear correlation experiments (COSY, HMQC and HMBC). The high crowding of methylene resonances precludes the accurate determination of ^{1}H chemical shifts and proton–proton couplings from one-dimensional measurements. These proton resonances, therefore, were assigned from the slices of the chemical shift heteronuclear correlation (HMQC) diagrams. For other experimental details see refs [8, 9]. MS were recorded at 70 eV, direct inlet, EI mode.

Isolation procedures. Canarina canariensis (L.) Vatke was cultivated in a greenhouse at the Botanischer Garten der TH Darmstadt. Fresh leaves and stems were very briefly rinsed with $CHCl_3$: to dissolve the waxy coating. The solvent was evapd *in vacuo* and the remainder was chromatographed over silica gel, eluting with toluene and increasing quantities of MeCOEt ketone and MeOH. TLC on silica gel (100–140°) (petrol–toluene–MeCOEt 18:1:1) revealed several spots showing triterpenoid reactions after being sprayed with $MnCl_2$ reagent [10]. Three compounds were isolated in reasonable amounts and in a sufficiently pure state for spectroscopic analyses. Two of them were unambiguously identified as the known compounds, taraxerone, mp 234°, recrystallized from EtOH, and isomultiflorenol acetate, mp 222°, recrystallized from toluene–Me_2CO. A third one (compound **1**, mp 220°, recrystallized from EtOH) required more detailed analysis.

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