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HYDROPEROXYCYCLOARTANES FROM *TILLANDSIA RECURVATA*

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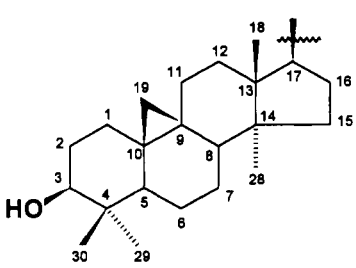
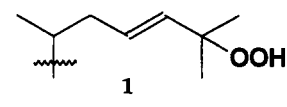
ABSTRACT.—25-Hydroperoxycycloart-23-en-3β-ol [**1**] and an epimeric mixture of 24-hydroperoxycycloart-25-en-3β-ol [**2**] have been isolated from *Tillandsia recurvata*. The structures of compounds **1** and **2** have been established by spectral analysis and chemical conversion to the corresponding diols.

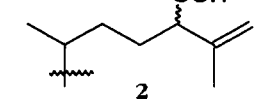
As part of our continuing interest in plants of the genus *Tillandsia* and their metabolites (1), we have examined an extract of *Tillandsia recurvata* (L.) L. (Bromeliaceae), an aerial plant endemic to Argentina. We now report the isolation of two new cycloartane triterpenes with an unusual hydroperoxide functional group, 25-hydroperoxycycloart-23-en-3β-ol [**1**] and 24-hydroperoxycycloart-25-en-3β-ol [**2**], from an extract of the fresh plant. In addition to these new compounds, we isolated and identified the known compounds cycloartanone, cycloartenone, 24-methylenecycloartanone, cycloartanol, cycloartenol, 24-methylenecycloartanol, lanostenol, lanosterol, and 24-ethylcholest-4-en-3-one.

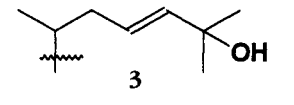
Compound **1** showed a molecular ion (eims, 20 eV) at *m/z* 458 corresponding to the molecular formula $C_{30}H_{50}O_3$. The ^{1}H-nmr spectrum displayed signals due to six tertiary [δ 0.81, 0.88, 0.97 (6H), 1.34 (6H)] and one secondary (δ 0.87, d, J=6.5 Hz) methyl groups. A pair of doublets at δ 0.33 and 0.56 (J=4.3 Hz) was indicative of a cyclopropane ring. The double doublet at δ 3.28 (J=10.6 and 5.1 Hz) was due to a proton attached to a carbon bearing a hydroxy group. Two trans olefinic protons appeared at δ 5.70 (ddd, J=15.7, 8.1, and 5.9 Hz) and 5.52 (d, J=15.7 Hz), and a broad singlet corresponding to an exchangeable proton appeared at δ 7.32. The ^{13}C-nmr spectrum showed signals for 30 carbon atoms. The multiplicity assignments were made by DEPT experiments. Four low-field signals were assigned to a double bond (δ 130.7 and 134.5), an oxygenated methine (δ 78.9), and an oxygenated quaternary carbon (δ 82.3). The high-field region showed ^{13}C-nmr resonances of the ABCD ring carbons similar to those reported for cycloartenol (2) and additional signals for a methine (δ 36.3), a methylene (δ 39.4), and three methyl carbons (δ 18.4, 24.3, and 24.4).

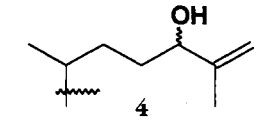
^{1}H-^{1}H COSY and long-range homonuclear COSY spectra exhibited correlations that allowed us to obtain the partial structure shown in Figure 1. Taking into

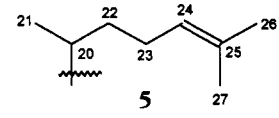












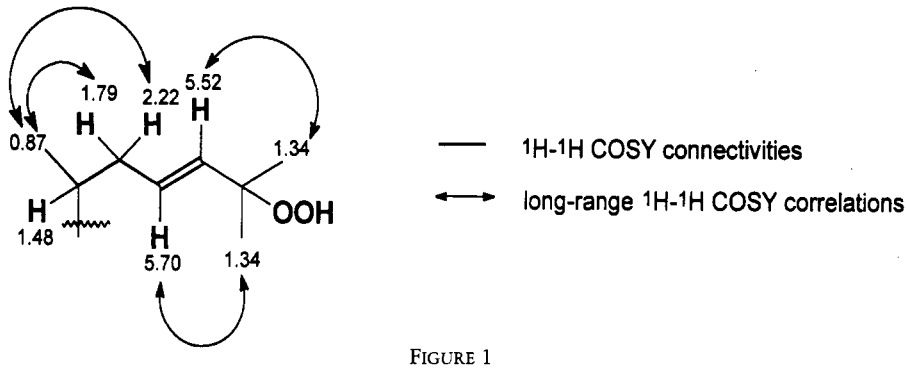


FIGURE 1

account the molecular formula, the substituent was identified as a hydroperoxide. Furthermore, a NOESY spectrum showed a nOe correlation between the exchangeable proton at δ 7.32 and H-24. Reduction of **1** with $LiAlH_4$ afforded **3** which was identified by direct comparison (tlc and ^{1}H-nmr) with an authentic sample (3,4).

Compound **2** exhibited a molecular ion at *m/z* 458 (eims, 20 eV). Comparison of the ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) with those of compound **1** suggested that compound **2** was also a 3β-hydroxycycloartane, isomeric with **1**. The most important differences between the ^{1}H-nmr spectra of **1** and **2** were the absence of the olefinic proton signals for H-23 and H-24 (δ 5.52 and 5.70), the methyl singlet for H-26, 27 (δ 1.34), and the presence of a two-proton broad singlet (δ 5.01), a triplet (δ 4.27, J=6.5 Hz), and a vinylic methyl group (δ 1.74) in the latter compound. The exchangeable proton signal was still present, but shifted downfield to δ 7.78. Differences between the ^{13}C-nmr spectra of the two compounds were consistent with a different location of the double bond and the hydroperoxide group in the side-chain. In the ^{13}C-nmr spectrum of **2**, in particular, all the signals corresponding to the side-chain were duplicated, suggesting that **2** was actually a mixture of two epimers (5). DEPT nmr experiments showed that the double bond was formed between a quaternary carbon and an sp^2 methylene. This fact and the presence of a vinylic methyl group suggested the presence of an isopropenyl group in the side-chain. The ^{1}H-^{1}H COSY nmr spectrum of **2** showed correlations between the broad singlet at δ 5.01 and the signals at δ 1.74 and 4.27, thus locating the

TABLE 1. ^{1}H-Nmr Chemical Shifts of **1** and **2** ($CDCl_3$).

Proton(s)	1	2
3	3.28 dd (J=10.5, 5.1 Hz)	3.28 dd (J=10.4, 5.0 Hz)
18	0.97 s	0.97 s
19	0.33 d (J=4.3 Hz)	0.33 d (J=4.2 Hz)
	0.56 d (J=4.3 Hz)	0.56 d (J=4.2 Hz)
21	0.87 d (J=6.5 Hz)	0.87 d (J=6.5 Hz)
23	5.70 ddd (J=15.7, 8.1, 5.9 Hz)	
24	5.52 d (J=15.7 Hz)	4.27 t (J=6.5 Hz)
26	1.34 s	5.01 br s
27	1.34 s	1.74 br s
28	0.88 s	0.89 s
29	0.97 s	0.97 s
30	0.81 s	0.81 s
HOO-	7.32 br s	7.78 br s

TABLE 2. ^{13}C-Nmr Chemical Shifts of **1** and **2** ($CDCl_3$).

Carbon	1	2
1	32.0	32.0
2	30.4	30.4
3	78.9	78.9
4	40.5	40.5
5	47.1	47.1
6	21.1	21.1
7	26.0	26.0
8	47.9	48.0
9	20.0	20.0
10	26.1	26.1
11	26.5	26.5
12	32.8	32.9
13	45.4	45.3
14	48.8	48.8
15	35.6	35.6
16	28.1	28.1
17	52.1	52.2, 52.1
18	18.1	18.0
19	29.9	29.9
20	36.3	36.0, 35.8
21	18.4	18.3, 18.2
22	39.4	32.0
23	130.7	27.6, 27.3
24	134.5	90.4, 90.2
25	82.3	143.9, 143.7
26	24.4[a]	114.5, 114.2
27	24.3[a]	17.2, 16.9
28	19.3	19.3
29	25.4	25.4
30	14.0	14.0

[a]Interchangeable signals.

hydroperoxy group vicinal to the terminal isopropenyl group of the side-chain. A NOESY spectrum showed an nOe correlation between the exchangeable proton and H-26 (δ 5.01). The ^{13}C-nmr chemical shifts of C-24 bearing the hydroperoxide substituent (δ 90.4, 90.2) were similar to those reported for 24-hydroperoxide-24-vinylcholesterol (6). Reduction of **2** with $LiAlH_4$ afforded **4**, which was identified by direct comparison (tlc and ^{1}H-nmr) with an authentic sample (3,4).

The known compounds, cycloartanone, cycloartenone, 24-methylenecycloartanone, cycloartanol, cycloartenol [**5**], 24-methylenecycloartanol, lanostenol, lanosterol, and 24-ethylcholest-4-en-3-one were identified by comparison of their spectral data (eims, ^{1}H-nmr, ^{13}C-nmr) with those reported in the literature (7–12).

The origin of compounds **1** and **2** perhaps may be understood as a naturally sensitized photooxygenation of cycloartenol [**5**] in the plant. The combination of molecular oxygen with olefins is a well-known phenomenon. The reaction involves the formation of an allylic hydroperoxide from an olefin by a process involving abstraction of an allylic proton along with migration of the carbon-carbon double bond (13,14). As there are two different types of allylic protons in **5**, two different products were obtained and as photooxygenation is non-stereoselective, an epimeric mixture was obtained in the case of compound **2**.

Other naturally occurring hydroperoxides have been reported from some plants (15,16) and tunicates (6) and their origin has been discussed, confirming that these compounds are not artifacts. A natural hydroperoxidation was also suggested to explain the biogenesis of some cycloartane derivatives (17). Taking into account that in this case the extraction was performed in darkness and the processing conditions were mild, it is apparent that this photooxygenation must take place in the plant itself. It is known that since $^1O_2$ is potentially damaging to chloroplast membrane lipids, proteins, and nucleic acids, protective systems are expected to exist in plants to quench these species (18), especially in plants like *T. recurvata*, with extreme exposure to air and sunlight due to growth on the upper parts of trees and on utility cables.

It is noteworthy that compounds **3** and **4**, which probably derive from **1** and **2**, have been reported from different plants (3,5), including *Tillandsia usneoides* (4). In all cases the extracts were obtained from dried plant material. Compounds **1** and **2** were probably not reported previously because they decompose during drying.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps are uncorrected. The Ft-ir spectra were recorded on a Nicolet Magna-IR 550 instrument. The uv spectra were taken on a Hewlett-Packard 8451 A diode-array spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Eims were taken on a Trio-2 quadrupole mass spectrometer (VG Biotech). Nmr spectra were recorded on a Bruker AC-200 instrument at 200.1 MHz for ^{1}H and 50.3 MHz for ^{13}C in $CDCl_3$ with TMS as internal standard.

PLANT MATERIAL.—*Tillandsia recurvata* was collected at Huerta Grande, Córdoba, Argentina, in November 1993 and was identified by Dra. Rosa Subils. A voucher specimen is located at the herbarium of the Museo Botánico de Córdoba [CORD], Argentina.

EXTRACTION AND ISOLATION.—Fresh plant material (1 kg) was extracted at room temperature in the dark with EtOH and then with CH_2Cl_2. H_2O was added to the EtOH extract and the solution was extracted with hexane. The hexane layer was combined with the CH_2Cl_2 extract and taken to dryness. The oily residue (9.5 g) was fractionated by dry-column flash chromatography on Si gel using hexane and mixtures of hexane/CH_2Cl_2 of increasing polarity. The fraction eluted with hexane-CH_2Cl_2 (1:1, 155 mg) was subjected to hplc (column, YMC C_{18}, 5 μm, 22.5×2.5 cm; eluent, MeOH) affording cycloartanone (15 mg), cycloartenone (3 mg), and 24-methylenecycloartanone (2 mg). The fraction eluted with CH_2Cl_2 (1.34 g) was fractionated by dry-column flash chromatography on reversed-phase (C_{18}) using mixtures of H_2O/MeOH and MeOH/CH_2Cl_2 of decreasing polarity. The fraction eluted with MeOH (545 mg) on hplc under the same conditions yielded cycloartanol (37 mg), lanostenol (42 mg), 24-methylenecycloartanol (15 mg), 24-ethylcholest-4-en-3-one (20 mg), cycloartenol (21 mg), 24-hydroperoxycycloart-25-en-3β-ol [**2**] (7 mg), 25-hydroperoxycycloart-23-en-3β-ol [**1**] (4 mg), and lanosterol (26 mg), some of which were re-purified by prep. tlc using CH_2Cl_2-EtOAc (9:1).

25-Hydroperoxycycloart-23-en-3β-ol [**1**].—White needles from hexane/Et_2O; mp 127–128°, $[\alpha]^{25}D$ +32° (*c*=0.43, $CHCl_3$); uv λ max ($CHCl_3$) 244 (ε 330) nm; ir (KBr) ν max 3380, 3266, 2938, 2867, 1462, 1377, 1370 cm^{-1}; eims (20 eV) *m/z* $[M]^+$ 458 (4), 440 (12), 424 (29), 409 (26), 393 (20), 365 (22), 342 (17), 339 (21), 315 (39), 297 (46), 255 (41), 175 (88), 121 (100); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

24-Hydroperoxycycloart-25-en-3β-ol [**2**].—White solid from MeOH/H_2O; mp 112–115°, $[\alpha]^{25}D$ +39° (*c*=0.10, $CHCl_3$); uv λ max ($CHCl_3$) 242 (ε 288) nm; ir (KBr) ν max 3433, 3276, 2926, 2855, 1466, 1375 cm^{-1}; eims (20 eV) *m/z* $[M]^+$ 458 (2), 440 (15), 424 (20), 409 (32), 381 (20), 315 (25), 297 (28), 255 (16), 175 (100); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

CONVERSION OF **1** TO **3** (OR **2** TO **4**).—To a solution of 2 mg of compound **1** (or **2**) in 0.5 ml anhydrous Et_2O, an excess of $LiAlH_4$ was added and the suspension was stirred for 2 h at room temperature. The reaction mixture was treated with MeOH; the salts were removed by filtration and the filtrate was evaporated to dryness. The crude mixture was chromatographed on Si gel (CH_2Cl_2-EtOAc, 9:1) affording 0.5 mg each of compounds **3** and **4**, which were identified by comparison with authentic samples (3,4).

The ^{1}H- and ^{13}C-nmr and eims spectra of cycloartanone, cycloartanol, and cycloartenol [**5**] were in full agreement with literature data (2,9,11,19) and with spectra of authentic samples. The ^{1}H- and ^{13}C-nmr and eims spectra of 24-methylenecycloartanol, lanostenol, lanosterol, and 24-ethylcholest-4-en-3-one were similar to those reported in the literature (7,10,12). The ^{1}H-nmr and eims spectra of 24-methylenecycloartanone and cycloartenone also agreed with those reported in the literature (7,8,10).

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