Two New 24-Isopropenyl-lanostanoids from *Tillandsia brachycaulos*[†]

Zulema Cantillo-Ciau[a], Gonzalo J. Mena-Rejón[a], Esther Quintero-Mármol[a],
Antonio Jiménez-Díaz[b], and Leovigildo Quijano[c]*

[a] Laboratorio de Química Orgánica de Investigación, Facultad de Química, Universidad Autónoma de Yucatán, Calle 41 No. 421, Col. Industrial, C. P. 97150, Mérida, Yucatán, México
[b] Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, La Laguna 38206, Tenerife, Islas Canarias, España
[c] Instituto de Química, UNAM, Circuito Exterior, Ciudad Universitaria, Coyoacán, 04510, México, D. F., México. Fax: (55)56162217. E-mail: quijano@servidor.unam.mx

* Author for correspondence and reprint requests

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The leaves of *Tillandsia brachycaulos* afforded two novel tetracyclic triterpenoids identified as (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-ol (**1**) and (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3-one (**2**), in addition to the known isopimaric acid (**3**) and chlorogenic acid (**4**). Their structures were elucidated on the basis of spectral analysis, including homo- and heteronuclear correlation NMR experiments (COSY, ROESY, HMQC and HMBC) and by comparison with data in the literature. The antimicrobial and antifungal activities were studied. The compounds did not show significant activity.

Key words: Tillandsia, Triterpenoids, NMR

Introduction

As part of our search for new bioactive (biologically active) compounds from plants of the Yucatán Peninsula, we have initiated the study of the genus *Tillandsia.* In a previous paper we reported on the isolation and structure elucidation of tillandsinone (**5**) (Cantillo-Ciau *et al.*, 2001), a triterpenoid of the cycloartane type with an isopropenyl group at C-24 of the side chain. Now we have undertaken the study of *T. brachycaulos*, collected at Dzibichaltun, Yucatán. Column chromatography of the hexane extract and ethyl acetate partition of the methanol extract of dried leaves of *T. brachycaulos* afforded in addition to the known isopimaric acid (**3**) (Wenkert and Buckwalter, 1972) and chlorogenic acid (**4**) (Pauli *et al.*, 1999), two novel triterpenoids of the 29-norlanostane type (**1**, **2**), with the same type of nonconventional side chain as in tillandsinone (**5**), isolated from *T. fasciculata.* All compounds were identified by extensive analysis of spectroscopic data and, and comparison with data in the literature. The antimicrobial and antifungal activities are described.

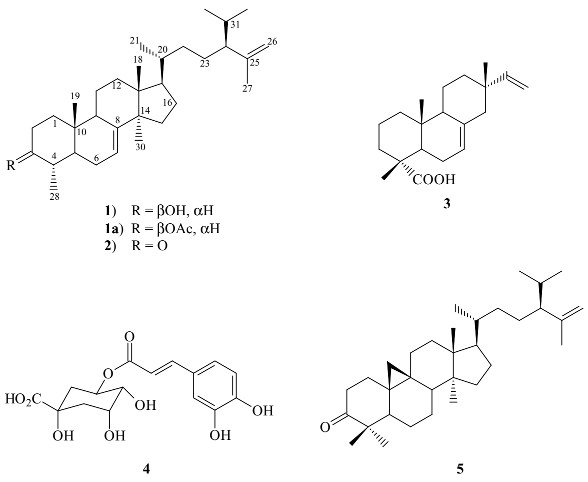

Fig. 1. **1**: (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-ol; **2**: (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3-one; **3**: isopimaric acid; **4**: chlorogenic acid; **5**: tillandsinone.

Results and Discussion

Compound **1**, $[\alpha]_D + 18.0°$, was obtained as colorless fine needles from CH_2Cl_2 with m.p. 168–170 °C and an IR absorption band at 3608 cm^{-1}, which suggested the presence of hydroxyl group in the molecule. The HR EIMS gave an accurate ion

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[†] In memory of Dr. Antonio González González[b] (1917–2002) and Dr. Alberto Sandoval Landázuri[c] (1918–2002).

Table I. ^{1}H NMR (500 MHz) spectral data for **1**, **1a** and **2** in $CDCl_3$.[a]

H	**1** δ *J* [Hz]	**1a** δ *J* [Hz]	**2** δ *J* [Hz]
1α	1.12 td (13.5, 3.5)	1.17 td (14.0, 4.0)	1.48 brtd (14.0, 4.0)
1β	1.82 m*	1.81 m*	2.13 ddd (13.0, 5.5, 2.5)
2α	1.80 m*	1.84 m*	2.29 ddd (14.0, 4.0, 3.0)
2β	1.45 m*	1.50 m*	2.48 brtd (14.0, 6.0)
3	3.11 td (11.5, 4.5)	4.40 td (11.0, 4.0)	– – –
4	1.30 m*	1.52 m*	2.30 dq (12.0, 6.0)
5	1.00 m*	1.07 td (11.5, 4.5)	1.41 td (11.5, 4.5)
6α	2.08 dddd (17.5, 6.0, 6.0, 2.5)	2.09 dddd (17.5, 6.0, 6.0, 2.5)	2.10 m*
6β	1.60 m*	1.60 m*	1.79 dddd (17.5, 11.5, 4.0, 1.5)
7	5.17 dt (5.5, 2.5)	5.17 dt (5.5, 2.0)	5.20 dt (5.5, 2.0)
9	2.0 m ($W_{1/2}$ ~ 25)	2.02 brm	2.08 m*
11	1.52 m*(2H)	1.50 m*(2H)	1.60 m* (2H)
12	1.66 m*(2H)	1.65 m*(2H)	1.67 m* (2H)
15α	1.22 m*	1.20 m*	1.24 m*
15β	1.57 m*	1.58 m*	1.60 m*
16α	1.95 dddd (13.5, 9.5, 9.5, 8.0)	1.92	1.94 dddd (13.5, 9.5, 9.5, 8.0)
16β	1.29 m*	1.24 m*	1.27 m*
17	1.51 m*	1.50 m*	1.52 m*
18	0.64 s	0.65 s	0.68 s
19	0.84 s	0.86 s	1.09 s
20	1.34 m*	1.32 m*	1.33 m*
21	0.89 d (7)	0.90 d (6.5)	0.90 d (6.5)
22a	0.81 m*	0.81 m*	0.81 m*
22b	1.35 m*	1.35 m*	1.35 m*
23a	1.01 m*	1.05 m*	1.03 m*
23b	1.60 m*	1.66 m*	1.62 m*
24	1.55 m*	1.52m*	1.53 m*
26a	4.61 brd (2.5)	4.61 brd (2.5)	4.61 brd (2.5)
26b	4.74 dq (2.5, 1.5)	4.75 dq (2.5, 1.5)	4.74 dq (2.5, 1.5)
27	1.57 brs	1.56 brs	1.57 brs
28	0.98 d (6.7)	0.85 d (6.5)	1.00 d (6.0)
30	0.97 s	0.98 pseudo d (0.5)	0.97 pseudo d (0.5)
31	1.53 m*	1.52 m*	1.52
32	0.90 d (6.2)	0.91 d (6)	0.91 d (6)
33	0.80 d (6.2)	0.81 d (6)	0.80 d (6)

[a] Assignments confirmed by COSY, NOESY and HMQC experiments. **1a**. Ac: δ_H 2.05 s.

* Overlapped multiplet.

peak at *m/z* 454.41764 corresponding to the molecular formula $C_{32}H_{54}O$ (calcd. 454.41747) which was also in good agreement with the ^{13}C NMR and DEPT spectral data. The ^{1}H and ^{13}C NMR spectra of compound **1** (Tables I and II) indicated the presence of eight methyl groups in the molecule. Three sharp proton singlet signals at δ 0.64, 0.84, and 0.97 (δ_C 16.0, 13.5 and 24.7, respectively) indicating the presence of three angular methyl groups, four secondary methyl group doublets at δ 0.80 (J = 6.2 Hz), 0.89 (J = 7.0 Hz), 0.90 (J = 6.2 Hz), and 0.98 (J = 6.7 Hz) (δ_C 21.5, 19.3, 20.8 and 15.0, respectively), and a vinyl methyl broad singlet at δ 1.57 (δ_C 18.9). The ^{1}H NMR spectrum also showed signals for three olefinic protons at δ 4.61, 4.74 due to a terminal methylene and δ 5.17 due to a trisubstituted double bond, as well as a methine proton attached to a carbon bearing a hydroxyl group (δ 3.11, td, J = 10.9 and 4.3 Hz). The ^{13}C NMR spectrum and DEPT experiments with **1** indicated the presence of 32 carbon atoms, which accounted for eight methyl groups, ten methylenes, including an olefinic one (δ 111.9), nine methines, one of them bearing a hydroxyl group (δ 76.2), and five quaternary carbon atoms, two of the latter being olefinic ones (δ 145.2 and 147.4). A comparison between the ^{1}H and ^{13}C NMR spectral data of **1** with those of tilladsinone

Table II. ^{13}C NMR (75 MHz) spectral data for **1**, **1a** and **2** in $CDCl_3$.[a]

C	**1**	HMBC	**1a**	**2**	HMBC
1	37.0 t	H-19	36.7	39.7 t	H-2, H-19
2	31.0 t		27.2	38.0 t	
3	76.2 d	H-1, H-2, H-4, H-5	78.5	213.4 s	H-1, H-2, H-4, H-28
4	40.3 d	H-28	37.1	45.7 d	H-28
5	46.8 d	H-1, H-4, H-6, H-7, H-19, H-28	46.8	50.4 d	H-4, H-6, H-7, H-19, H-28
6	26.8[c] t		26.81[c]	28.2[c] t	H-7, H-4
7	116.1 d	H-6, H-9	115.9	115.8 d	H-6
8	145.2 s	H-6, H-9, H-30	145.3	145.4 s	H-6, H-30
9	44.8 d	H-19	44.7	44.4 d	H-7, H-19
10	35.0 s	H-19	34.9	35.3 s	H-19
11	20.4 t		20.4	20.5 t	
12	32.2[b] t	H-18	32.14[b]	32.09[b] t	H-18
13	44.3 s	H-16, H-18, H-30	44.3	44.3 s	H-16, H-18
14	52.0 s	H-7, H-15, H-16, H-18, H-30	52.0	52.0 s	H-7, H-15, H-18, H-30
15	32.1[b] t	H-30	32.11[b]	32.06[b] t	
16	27.5 t		27.5	27.5 t	
17	50.7 d	H-15, H-16, H-18, H-21	50.7	50.6 d	H-18, H-21
18	16.0 q		16.1	16.1 q	
19	13.5 q		13.4	13.1 q	
20	36.9 d		37.0	36.9 d	H-21
21	19.3 q		19.3	19.3 q	
22	34.5 t		34.5	34.5 t	H-21
23	26.7[c] t		26.76[c]	26.7[c] t	
24	55.5 d	H-26, H-27, H-32, H-33	53.5	55.5 s	H-26, H-27, H-32, H-33
25	147.4 s	H-27	147.4	147.3 s	H-27
26	111.9 t	H-27	111.9	111.9 t	H-27
27	18.9 q		19.0	18.9 q	H-26
28	15.0 q		15.1	11.3 q	
30	24.7 q		24.8	24.7 q	
31	30.2 d	H-32, H-33	30.3	30.2 d	H-32, H-33
32	20.8 q	H-33	20.9	20.8 q	
33	21.5 q	H-32	21.5	21.5 q	

[a] Assignments confirmed by DEPT, HMQC and HMBC experiments. **1a**, Ac: δ_C 21.4 q, 170.9 s.

[b, c] Signals in the same column may be interchanged.

(**5**) (Cantillo-Ciau *et al.*, 2001) revealed that the methyl protons and the olefinic methylene signals, as well as the carbon signals of the side chain of the two molecules were almost identical. On the other hand the ^{1}H and ^{13}C chemical shifts of the cyclic part of **1** were also very close to those reported for (24*E*)-24-ethyl-29-*nor*-5α-lanosta-7,24(31)-dien-3β-yl acetate (Akihisa *et al.*, 1996). The combined data suggested that compound **1** was 24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-ol.

The HMBC spectrum confirmed the above assumption, since correlations were observed between H-7 (δ_H 5.17) and a methine signal at δ_C 46.8 (C-5), a methylene signal at δ_C 26.7 (C-6), and signals of two quaternary carbon atoms at δ_C 44.8 (C-9) and 52.0 (C-14) and between H-3 (δ_H 3.11) and a methyl signal at δ_C 15.0 (C-28). Additionally, the methyl signal at δ_H 0.64 (Me-18) showed correlations with a methylene signal at δ_C 32.1 (C-12), a methine signal at δ_C 50.7 (C-17), and two quaternary carbon signals at δ_C 44.3 (C-13) and 52.0 (C-14); the methyl signal at δ_H 0.84 (Me-19) showed correlations with a methylene signal at δ_C 37.0 (C-1), a methine signal at δ_C 44.8 (C-9) and two quaternary carbon signals at δ_C 46.8 (C-5) and 35.0 (C-10). The methyl signal at δ_H 0.97 (Me-30) showed correlations with a methylene signal at δ_C 32.2 (C-15) and three quaternary carbon signals, an olefinic one at δ_C 145.2 (C-8) and C-13 and C-14.

The HMBC experiments also confirmed the presence of the isopropyl group at C-24, since long range couplings were observed between the C-24 (δ 55.5) and the protons of the vinyl methyl (H-27,

δ 1.57), the terminal methylene (H-26, δ 4.61 and 4.74), and the methyl group doublets H-32 and H-33 (δ 0.90 and 0.80) (see Fig. 1).

Concerning the stereochemistry of the hydroxyl and methyl groups at C-3 and C-4, the multiplicity and coupling constants of H-3 (td, $J = 10.9$, 4.3 Hz) indicated that H-3 and H-4 are in the α-*axial* and β-*axial* orientations, respectively, therefore the methyl group at C-4 must be in the β-*equatorial* direction. Since the ^{1}H and ^{13}C NMR spectral data for the side chain of compound **1** and tillandsinone (**5**) (Cantillo-Ciau *et al.*, 2001) were nearly identical, this strongly suggests that the stereochemistry at C-24 must be the same. Therefore the structure of **1** was established as (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-ol.

Acetylation of **1**, gave the corresponding acetate **1a**. All the spectral data of acetate **1a** were in good agreement with the proposed structure of **1**.

Compound **2** was obtained as colorless crystals from EtOAc with m.p. 171–172 °C. The IR spectrum showed an absorption band at 1705 cm^{-1}, indicating the presence of a keto group in the molecule. The HR-EIMS showed an accurate molecular ion peak at *m/z* 452.40612, that is two amu less than compound **1**, suggesting a molecular formula of $C_{32}H_{52}O$ (calcd. 452.40182). The ^{1}H and ^{13}C NMR spectral data of **2** were very similar to those of **1**, except for the absence of the C-3 methine signals and the differences in chemical shifts associated with the presence of the keto group at C-3. The presence of the ketone at C-3 was evident in the IR and the ^{13}C NMR spectrum since it showed a carbonyl signal at δ 213.4, which showed cross-peaks with protons at C-1, C-2, C-4 and the methyl group at C-4 in the HMBC spectrum. All these data suggested that compound **2** must be the oxidation product of **1**. Jones oxidation of **1** afforded the product which was identical in all respects with compound **2**. Thus the structure of **2** was established as (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3-one.

The known compounds were identified as isopimaric acid (**3**) (Wenkert and Buckwalter, 1972) and chlorogenic acid (**4**) (Pauli *et al.*, 1999) by comparison of their ^{1}H and ^{13}C NMR spectral data with those reported in the literature (Fig. 1).

As a continuation of a program oriented towards the discovery of bioactive natural products, the isolated compounds were evaluated for their antimicrobial and antifungal activities. The tested compounds showed no significant activity.

Experimental

General experimental procedures

Melting points were determined on a Fisher-Jones type apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions on a Rudolph Research Autopol IV polarimeter. IR spectra were recorded on KBr disks on a Nicolet Magna 750 Fourier transform IR spectrometer. EIMS were obtained on a Hewlett-Packard 5970 series II gas chromatograph as injection system. HREIMS were done on a VG Micromass LTD-ZAB-2F spectrometer at 70 eV. NMR spectra were recorded on Bruker Avance 400 and Varian Unity Pluss500 spectrometers in $CDCl_3$ solutions with TMS as internal standard; chemical shifts are recorded in δ values.

Plant material

Tillandsia brachycaulos Schltdl was collected at Dzibichaltun located at 21°05′25″ N; 89°35′52″W of Mérida, Yucatán (México) in March 2001 and authenticated by Francisco Chi-May and Ulises de la Cruz. A voucher specimen has been deposited at the herbarium "Ü najil tikin xiw", Centro de Investigación Científica de Yucatán (CICY) (voucher F. Chi-May, Zulema Cantillo & Ulises de la Cruz 4).

Extraction and isolation

Dried and ground leaves of *T. brachycaulos* (1.5 kg) were extracted with hexane and MeOH in a Soxhlet apparatus to give green dark residues (*ca.* 32 and 82.6 g, respectively). The hexane residue (30 g) was chromatographed on a silica gel column (8 × 10 cm), eluted with hexane, mixtures of hexane-EtOAc (9.5:0.5, 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 5.5:4.5, 4:6) and EtOAc. Eluates (49) were collected, monitored by TLC, and combined in seven major fractions (A1–A7). Fraction A2 (2.2 g) was subjected to Sephadex LH-20 column chromatography eluted with a mixture of hexane-$CHCl_3$-MeOH (2:1:1 v/v/v) to yield 16 fractions, that were combined in 6 secondary fractions according with their TLC profiles. Fraction 2 (390 mg) was rechromatographed on a silica gel column (2 × 12 cm) using mixtures of hexane-Et_2O (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 50:50) and EtOAc as eluents. Eluates (100) were collected, monitored by TLC and combined giving 10 new fractions. Fraction 2 contained 13 mg of (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-ol (**1**). Fraction 8 (40 mg) was purified by TLC on silica gel with hexane-$CHCl_3$ (1:1, developed three times) giving 21.6 mg of (24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3-one (**2**). Fraction A4 (2.61 g) was subjected to column chromatography on Sephadex LH-20 as described before. Eluates (23) were collected, monitored by TLC and combined in 5 fractions. Fraction 5 (61.1 mg) was further purified by preparative TLC on silica gel with hexane-Et_2O (1:1, developed three times) giving 10 mg of isopimaric acid (**3**). Fraction A5 (1.84 g) was also chromatographed on Sephadex LH-20 as described above. Fraction 4 (20.4 mg) gave a mixture of sterols identified by GC-MS as stigmasterol, campesterol and β-sitosterol.

The MeOH extract (82.6 g) was fractionated by successive partitions with CH_2Cl_2 and EtOAc. The EtOAc soluble fraction (9.7 g) was chromatographed on a silica gel column (4.5 × 12 cm) using mixtures of hexane-EtOAc (90:10, 80:20, 60:40, 40:60, 20:80, 0:100) and mixtures of EtOAc-MeOH (97:3, 90:10, 50:50, 0:100) as eluants. Eluates (38) were collected, monitored by TLC and combined in 14 fractions. Fraction 10 (1.63 g) after column chromatography on Sephadex LH-20 using $CHCl_3$-MeOH (1:1) afforded 31.2 mg of the 15-O-ferulate of quinic acid (chlorogenic acid) (**4**).

(24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-ol (**1**). Colorless fine needles, m.p. 168–170 °C. – $[\alpha]_D^{25}$ + 18.0 ° (*c* 0.1, $CHCl_3$). – IR ($CHCl_3$) ν_{max} = 3607, 1643, 1602, 1023, 928, 892 cm^{-1}. – HREIMS *m/z* = 454.41764 $[M]^+$ (7) (calcd. for $C_{32}H_{54}O$, 454.41747), 436 $[M\text{-}H_2O]^+$ (78), 421 $[M\text{-}Me\text{-}H_2O]^+$ (100), 393 $[M\text{-}H_2O\text{-}C_3H_7]^+$ (7), 324 $[M\text{-}H_2O\text{-}Me\text{-}C_7H_{13}]^+$ (17), 309 $[M\text{-}H_2O\text{-}Me\text{-}C_8H_{17}\text{-}H]^+$ (24), 283 $[M\text{-}H_2O\text{-}C_{11}H_{21}(SC)]^+$ (24), 281 $[M\text{-}H_2O\text{-}SC\text{-}2H]^+$ (40), 256 $[M\text{-}H_2O\text{-}SC\text{-}C_2H_3 \text{ from ring D}]^+$ (24), 241 $[M\text{-}H_2O\text{-}Me\text{-}C_{13}H_{24}\text{-}SC\text{-}C_2H_3)]^+$, 227 $[M\text{-}H_2O\text{-}Me\text{-}SC\text{-}C_3H_5 \text{ (ring D)}]^+$ (40), 215 (28), 95 (72), 81 (47), 69 (88), 55 (77). – ^1H and ^{13}C NMR: Tables I and II.

(24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3-one (**2**). Colorless crystals, m.p. 171–172 °C. – $[\alpha]_D^{25}$ + 4.0° (*c* 0.18, $CHCl_3$). – IR ($CHCl_3$) ν_{max} = 1704, 1643, 1452, 1381, 925, 892 cm^{-1}. – HREIMS *m/z* = 452.40612 $[M]^+$ (58) (calcd. for $C_{32}H_{52}O$, 452.40182), 437 $[M\text{-}Me]^+$ (98), 409 $[M\text{-}C_3H_7]^+$ (6), 354 $[M\text{-}C_7H_{13}\text{-}H]^+$ (7), 340 $[M\text{-}Me\text{-}C_7H_{13}]^+$ (28), 339 $[M\text{-}Me\text{-}C_7H_{13}\text{-}H]^+$ (45), 325 $[M\text{-}Me\text{-}C_8H_{15}\text{-}H]^+$ (46), 311 $[M\text{-}Me\text{-}C_9H_{17}\text{-}H]^+$ (40), 299 $[M\text{-}C_{11}H_{21}(SC)]^+$ (28), 297 $[M\text{-}SC\text{-}2H]^+$ (46), 285 $[M\text{-}SC\text{-}CH_2]^+$ (53), 283 $[M\text{-}SC\text{-}2H\text{-}CH_2]^+$ (37), 272 $[M\text{-}SC\text{-}C_2H_3]^+$ (39), 257 $[M\text{-}Me\text{-}SC\text{-}C_2H_3]^+$ (78), 243 $[M\text{-}Me\text{-}SC\text{-}C_3H_5 \text{ (ring D)}]^+$ (35), 231 $[C_{16}H_{23}O]^+$ (67),180 (100), 137 (63), 95 (78), 69 (98), 55 (98). – ^1H and ^{13}C NMR: Tables I and II.

(24*S*)-24-isopropenyl-29-*nor*-5α-lanosta-7-en-3β-yl acetate (**1a**). A sample of **1** (5 mg) was acetylated with Ac_2O/pyridine in the usual manner, to give 4.8 mg of **1a** as gummy material. – IR ($CHCl_3$) ν_{max} = 1722, 1644, 1469, 1456, 1380, 1027, 975, 892 cm^{-1}. – EIMS *m/z* = 496 $[M]^+$ (62), 481 $[M\text{-}Me]^+$ (100), 436 $[M\text{-}AcOH]^+$ (6), 421 $[M\text{-}Me\text{-}AcOH]^+$(39), 398 $[M\text{-}C_7H_{13}\text{-}H]^+$ (12), 383 $[M\text{-}H\text{-}Me\text{-}C_7H_{13}\text{-}H]^+$ (17), 369 $[M\text{-}Me\text{-}C_8H_{15}\text{-}H]^+$ (14), 355 $[M\text{-}Me\text{-}C_9H_{17}\text{-}H]^+$ (14), 341 $[M\text{-}SC\text{-}2H]^+$(20), 316 $[M\text{-}SC\text{-}C_2H_3 \text{ from ring D}]^+$ (25), 301 $[M\text{-}Me\text{-}SC\text{-}C_2H_3]^+$ (22), 269 $[M\text{-}Me\text{-}AcOH\text{-}SC]^+$ (43), 256 $[M\text{-}AcOH\text{-}SC\text{-}C_2H_3 \text{ from ring D}]^+$ (44), 241 $[M\text{-}Me\text{-}AcOH\text{-}SC\text{-}C_2H_3]^+$, 180 (31), 95 (57), 81 (38), 69 (50), 55 (52). – ^1H and ^{13}C-NMR: Tables I and II.

Oxidation of 1

A sample of 100 mg of **1**, was oxidized with Jones' reagent in acetone and treated in the usual manner, the reaction product was purified by CC on silica gel, to give the oxidation product which was identical in all respects with compound **2**.

Antimicrobial assay

Isolated compounds were screened, using the disc-diffusion method, against *Staphylococcus aureus* (6338), *Bacillus subtilis* (6633), *Streptococcus agalactiae* (260), *Escherichia coli* (10536), *Pseudomonas aeruginosa* (10231), *Klebsiella pneumoniae* (4209), *Shigella flexneri* serotype 4 (9748), *Candida albicans* (752), *Sacharomyces cerevisiae* (287), *Aspergillus niger* (16888) and *Tricophyton mentagrophytes* (4807); all of them were purchased from the American Type Culture Collection. Diluted bacterial cultures were spread on sterile Muller-Hinton agar plates, after which

6-mm-diameter-discs, impregnated with 10 μl of a 5% (w/v) chloroformic solution of each compound, were placed on the plates. The plates were incubated for 24 h at 37 °C under aerobic conditions and the diameter of the inhibition zone around each disc was then measured and recorded. Amikacin (0.03 mg/μl), nystatin (50 IU/μl) and itraconazole (0.025 mg/μl) were used as positive control for bacteria, yeast and fungi, respectively; chloroform was used as negative control. All determinations were performed in triplicate.

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