

SHORT REPORTS

TRIXANE DERIVATIVES FROM *TRIXIS PRAESTANS*

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Key Word Index—*Trixis praestans*; Mutisieae; Compositae; trixanes; sesquiterpenes.

Abstract—Chemical Investigation of *Trixis praestans* resulted in isolation of several new sesquiterpene diesters of the trixane type as well as common plant constituents.

INTRODUCTION

Sesquiterpenes based on the unusual carbon skeleton 1 have been isolated from several *Trixis* species and appear to be characteristic secondary metabolites of Mutisieae, Subtribe Nassauviinae [1-5]. In the present report we describe our study of *Trixis praestans* (Velloso) Cabrera which has led to the identification of new representatives of the system, i.e. the pairs of C-12 epimers 2a,a', 2b,b', 2c,c' and 2d,d', as well as other well-known plant constituents. Bohlmann and coworkers [1-5] have used the term 'isocedrane' for describing carbon skeleton 1. However, it has been pointed out [6] that this term is inappropriate and we therefore propose the trivial name 'trixane' for the basic carbon skeleton.

RESULTS AND DISCUSSION

The sets of epimers 2a,a', 2b,b', 2c,c' and 2d,d' which were difficult to purify (2c,c' was only seen in admixture with 2a,a' or 2b,b') and decomposed easily at room temperature, particularly when traces of acid were present, due to cleavage of the acylated hemiacetal functions, were clearly analogues of two sets of C-12 epimers 2g,g' and 2h,h' isolated earlier [2] from *T. wrightii* and *T. inula*.

Extensive decoupling of the ¹H NMR spectra led to the assignments in Table 1 with, for example in the case of 2a,a', duplicate signals, each of equal intensity, for H-4 (δ 4.41 and 4.33, *t*'s *J* = 2.5 Hz) H-9 (5.25 and 5.24, *t*'s, *J* = 6 Hz), H-12 (5.02 and 4.99 *br*), H-13 (1.24 and 1.20, Me singlets), H-14 (5.57 and 5.55 *d*, *J* = 8 Hz) and H-15 (6.35 and 6.33 *s*). On the basis of models one would also expect duplicate signals for H-2, H-3a,b and H-10 and while accurate chemical shifts of most of the upfield signals were difficult to determine due to superposition of signals (see Table 1) we found that two resonances with widely different shifts corresponded to H-10, one at δ 2.76 for the epimer (or epimers—see below) with H-9 at 5.22, presumably the epimer with C-12 OH oriented toward H-10, and one near 2.4 for the epimer with H-9 at 5.25. The latter epimer has H-4 at δ 4.33 and H-3b at 2.89, while the former has H-4 at 4.41 and H-3b at 2.90.

The remaining source of uncertainty was the distribution of the two different acyl functions (acetate on the one hand and angelate, isovalerate, 2-methylbutanoate and senecioate on the other) over C-9 and C-14. It is not clear on what basis the German authors [2] preferred placement of the 2-methylbutanoyl group of their two epimer pairs on C-14 and the senecioyloxy, resp. isovaleryloxy group on C-9 instead of the reverse.† The following observation suggests that in fact 2a,a'-2d,d' may represent not only C-12 epimeric pairs but also mixtures of C-9 and C-14 acetates. Thus, acetylation of 2a,a' and 2b,b' furnished unequal amounts of two C-12 acetates, 2e and 2e', from 2a,a', and two, 2f and 2f', from 2b,b', which could be separated by HPLC, a circumstance which facilitated analysis of their NMR spectra (Table 1). Compounds 2e,e' and 2f,f' were at first thought to be pairs of C-12 epimers; however, both acetates from 2a,a', and both acetates from 2b,b', exhibited almost identical ¹H NMR spectra with the H-10 signal invariably located near δ 2.80. Hence the C-12 stereochemistry of the components of each pair 2e,e' and 2f,f' must be assumed to be identical.

†In theory a distinction between the two possibilities for 2a,a'-2d,d' and 2g,g' might be achieved by observing the effect on the C=O signals (near δ 170 for acetate carbonyls, near δ 167 for the angeloyl and senecioyl carbonyls and approx. δ 176 for the isovaleryl and 2-methylbutanoyl carbonyl) by selectively irradiating H-9 or H-14. In our case, smallness of sample and the ease with which 2a,a'-2d,d' decomposed on standing prevented determination of the ¹³C NMR spectra and thus resolution of the ambiguity.

Table 1. ^1H NMR spectra of compounds **2a,a'-2f,f'** (270 MHz, CDCl_3)

H	2a,a' †	2b,b' ‡§	2d,d'	2e (major)†	2e' (minor)†	2f (major)‡	2f (minor)‡
1a	1.38 br d (11) 1.32 br d 1.36 br d	1.36 br d 1.28 br d 1.28 br d	1.36 br d 1.28 br d 1.28 br d	1.32 br d (11, 1.5)	~1.3	1.31 br d	~1.3
1b	2.7 m	2.7 m	2.7 m	2.71 br dd (11, 6)	2.71 m	2.71 m	2.71 m
2	~2.15 m	~2.15 m	~2.14 m	~2.2 m	~2.2 m	~2.2 m	~2.2 m
3a	~1.5 m	~1.5 m	~1.5 m	1.56 br dd (14, 3)	~1.5 m	1.55 br dd	~1.5 m
3b	2.90 dt (11, 2.5) 2.89 dt	2.90 dt 2.89 dt	2.90 dt 2.89 dt	2.47 m	~2.45 m	~2.44 m	~2.42 m
4	4.41 t (2.5) 4.33 t	4.39 t 4.31 t	4.39 t 4.31 t	4.48 t(2)	4.38 br	4.48 t	4.35 br
7	~2.48 m	~2.42 m	~2.46 m	~2.44 m	~2.45 m	~2.44 m	~2.42 m
8a	~2.4 m	~2.4 m	~2.4 m	~2.2 m	~2.2 m	~2.2 m	~2.2 m
8b	~1.95 m	~1.95 m	~1.95 m	obsc.	obsc.	obsc.	obsc.
9	5.22 t (6) 5.25 t	5.24 t 5.25 t	5.23 t 5.22 t	5.27 t	5.25 t	5.27 t	5.23 t
10	2.76 br d (6) ~2.37 m	2.75 d ~2.38 m	2.75 d ~2.38 m	2.82 dd (6, 1.5)	2.78 dd	2.80 dd	2.80 dd
12	5.01 s, 4.98 s	5.02 s, 4.98 s	5.02 s, 4.98 s	5.88 s	5.98 s	5.88 s	5.96 s
13*	1.24 s, 1.20 s	1.24 s, 1.19 s	1.25 s, 1.20 s	1.13 s	1.15 s	1.12 s	1.14 s
14	5.57 d, 5.55 d (8)	5.48 d, 5.45 d	5.52 d, 5.49 d	5.59 d	5.55 d	5.50 d	5.46 d
15	6.35 s, 6.33 s	6.32 s, 6.31 s	6.34 s, 6.32 s	6.37 s	6.35 s	6.33 s	6.35 s
Ac*	2.10 s, 2.09 s	2.09 s, 2.08 s	2.08 s, 2.07 s	2.15 s	2.11 s	2.11	2.11 s
				2.11 s	2.10 s	2.09	2.10 s

*Intensity three protons.

†Ang 6.18 *qq* (7, 1.5) (H-3'), 2.01 *dq* (7, 1.5) (H-4')*, 1.92 *br* (H-5')*.‡*i*-Val ~2.25 m (H-2'), ~1.5 m (H-3'), 0.98 *d* (H-4', 5') (6ps').§ 2-MeBu ~2.4 m (H-2'), 1.7 *ddq* (7) (H-3'), ~1.5 m (H-3'), 1.17 *d* (H-5')*, 0.93 *t* (H-4')*.||Sen 5.70 *br* *t* (1) (H-2') 2.19 *d* (1) (H-4')*, 1.93 *d* (1) (H-5')*.

A plausible biogenetic route to the trixane system which utilizes a guaiane precursor is outlined in Scheme. The proposal is supported by the co-occurrence of guaienes, cyperenes and trixanes in a *Moscharia* species [4].

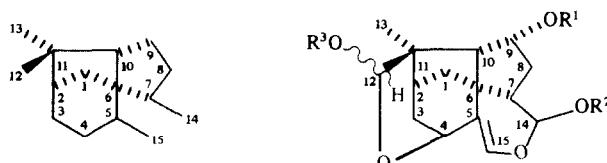
EXPERIMENTAL

General. For separation of mixtures Waters HPLC equipment (M45 pump, U6K injector with 2 ml loop and R-401 differential refractometer) was used. The column employed was an ALTEX

Ultrasphere ODS column (5 μm , 10 mm, inner diameter \times 25 cm). Retention times were measured from the injection point.

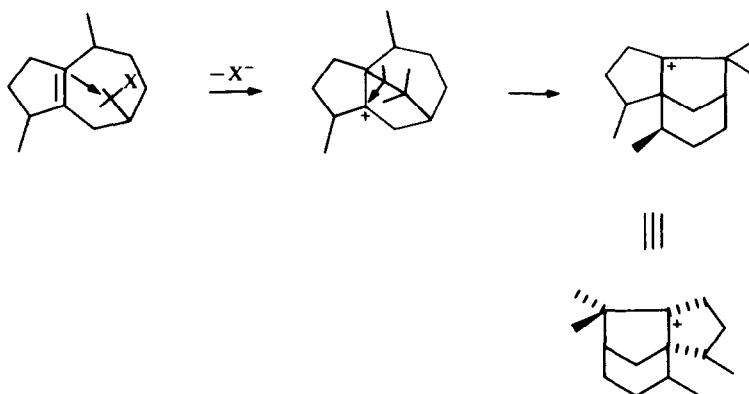
Plant material. Aerial parts of *Trixis paeastans* (Velloso) Cabrera were collected in July 1985 at the flowering stage at km. 5, Route 11, Departamento Paraná, Entre Ríos Province, Argentina, by Mr. J. M. Retamal, IPNAYS, Facultad de Ingeniería Química, Universidad Nacional del Litoral and identified by Ing. J. M. Jozami (voucher no. 280, IPNAYS). Flowers and leaves were processed separately.

Extraction of *T. paeastans*. Flowers (500 g) were extracted



1

2a,a' $\text{R}^1, \text{R}^2 = \text{Ac, Ang, R}^3 = \text{H}$ **2b,b'** $\text{R}^1, \text{R}^2 = \text{Ac, } i\text{-Val, R}^3 = \text{H}$ **2c,c'** $\text{R}^1, \text{R}^2 = \text{Ac, 2-MeBu, R}^3 = \text{H}$ **2d,d'** $\text{R}^1, \text{R}^2 = \text{Ac, Sen, R}^3 = \text{H}$ **2e,e'** $\text{R}^1, \text{R}^2 = \text{Ac, Ang, R}^3 = \text{Ac}$ **2f,f'** $\text{R}^1, \text{R}^2 = \text{Ac, } i\text{-Val, R}^3 = \text{Ac}$ **2g,g'** $\text{R}^1 = \text{Sen, R}^2 = \text{2-MeBu, R}^3 = \text{H}$ **2h,h'** $\text{R}^1 = i\text{-Val, R}^2 = \text{2-MeBu, R}^3$



with 2×6 l of CHCl_3 at room temp for 7 days to give 26 g of extract which was suspended in 350 ml of EtOH at 50–55°, diluted with 250 ml of H_2O and extracted successively with hexane (3×350 ml) and CHCl_3 (3×300 ml). Evaporation of the hexane fraction gave 19.6 g of residue, a portion of which (15 g) was chromatographed over silica gel (460 g) using hexane and increasing amounts of Et_2O , all fractions being monitored by TLC. Fractions with higher R_f than β -amyrin (hexane– EtOAc 5:2) were combined to yield 8.7 g of waxy material which was not processed further. Fractions with the same R_f as β -amyrin were combined to give 1.6 g of residue which was saponified with dil. KOH . The unsaponified material (1.13 g) was purified by flash chromatography (florisil, hexane– Et_2O 5:2). Reversed-phase chromatography (eluting solvent MeOH , flow rate 3 ml/min) of a small portion (75 mg) afforded lupeol (25 mg), β -amyrin (15 mg), germanicol (18 mg) and *n*-triacontanol (3 mg). Lupeol and β -amyrin were identified by mp, ^1H NMR and cojunction of authentic material; germanicol by mp (178–180°), ^1H NMR, MS, and mp of its acetate (275–276°), *n*-triacontanol by ^1H NMR and MS. Fractions with an R_f close to cholesterol were combined and separated by HPLC to give 0.8 mg of isofucosterol, 10 mg of stigmasterol and 16 mg of sitosterol, all being characterized by cojunction of authentic material, GC retention time, mp and ^1H NMR.

The CHCl_3 extract gave a residue (5.1 g) which was purified by CC (silica gel, 150 g) using CHCl_3 and increasing amounts of Et_2O (0–35%). Thirty-three fractions were collected. Fr. 18–22 which showed one major spot were combined and separated by HPLC ($\text{MeOH–H}_2\text{O}$ 2:1, flow rate 2 ml/min) to give 9 mg of **2a,a'** as a 1:1 mixture of C-12 epimers (R_f 35 min) and 6 mg of **2b,b'** as a 1:1 mixture of C-12 epimers (R_f 43 min) containing a small amount of **2c,c'** (NMR analysis). Fractions 23–27 were combined and separated by HPLC to give 4.6 mg of **2d,d'** as a 1:1 mixture of epimers.

Extraction of leaves of *T. praestans* (500 g) and work-up in the same manner furnished 19.9 g of hexane extract and 6.5 g of CHCl_3 extract. The hexane extract after further purification as described in above gave the same triterpene and sterol constituents as the flowering parts. The CHCl_3 extract showed one major spot on TLC (R_f 0.42, $\text{CHCl}_3\text{–EtOAc}$ 2:1) and only traces of more polar substances. CC (silica gel, $\text{CHCl}_3\text{–Et}_2\text{O}$ 2:1) gave 3.9 g of material which HPLC ($\text{MeOH–H}_2\text{O}$ 2:1, 2 ml/min) resolved into four peaks in the ratio 3:4:5:2. Peak 3 (R_f 29 min) was identified as **2a,a'** (1:1 mixture of C-12 epimers) and peak 4 as a mixture of approximately equal amounts of **2b,b'** and **2c,c'**.

9(or 14)-Acetoxy-14 (or 9)-angelyloxy-4(12),14(15)-diepoxy-12-hydroxy-trix-5(15)-ene (2a,a'). The ^1H NMR spectrum (Table 1) showed this to be a 1:1 mixture of C-12 epimeric alcohols which decomposed easily on standing, mp 78–80° (without recrystallization); IR ν_{max} cm^{-1} : 3450, 1720–1740, 1665; MS m/z (rel. int.): 404 [M] $^+$ (0.7), 386 (0.4), 373 (2.7), 358 (0.3), 305 (10.8), 304 (100), 303 (43.8), 276 (7), 260 (31), 244 (9.9), 226 (18.6), 198 (1.8); ^1H NMR spectrum in Table 1.

Acetylation (Ac_2O , pyridine, 7 hr) gave solid material which on HPLC ($\text{MeOH–H}_2\text{O}$ 2:3, flow rate 3 ml/min) showed two peaks in the ratio 7:1. Collected separately they were identified as **2e** (R_f 40 min, major constituent, 8.8 mg, crystalline solid) and **2e'** (R_f 56 min, minor constituent, also a solid, 2.3 mg); ^1H NMR spectra in Table 1.

9(or 14)-Acetoxy-14 (or 9)-isovaleryloxy-4(12)-14(15)-diepoxy-12-hydroxytrix-5(15)-ene (2b,b') and **9(or 14)-acetoxy-14(or 9)-(2-methylbutanoyloxy)-4(12,14-(15)-diepoxy-12-hydroxy-5(15)-ene (2c,c')**. The solid material from the flowering parts (no mp taken) was mainly a 1:1 mixture of C-12 epimers **2b,b'** containing a small amount of the **2c,c'** epimers, (NMR analysis), which decomposed easily on standing; IR ν_{max} cm^{-1} : 3440, 1725–1745, 1660; MS m/z (rel. int.): 406 [M] $^+$ (1.1), 388 (0.5), 360 (1.4), 260 (24.9), 244 (5.2), 226 (15.4), 216 (1.3), 198 (1.4). The ^1H NMR spectrum of the material from the leaves, mp 61–62° (without recrystallization) showed it was a mixture consisting of approximately equal parts of **2b,b'** and **2c,c'**.

Acetylation of the epimeric mixture and separation in the manner described for **2a,a'** afforded as major product 11 mg of **2f**, mp 148–150° (without recrystallization) and as minor product 2.8 mg of **2f'** (solid, but mp not determined). ^1H NMR spectra which revealed that these fractions were essentially free of diacetoxy-(2-methylbutanoyloxy) analogues are listed in Table 1.

9(or 14)-Acetoxy-14 (or 9)-senecioyloxy-4(12),14(15)-diepoxy-12-hydroxytrix-5(15)-ene (2d,d'). The ^1H NMR spectrum (Table 1) showed this to be a 1:1 mixture of C-12 epimeric alcohols which decomposed easily on standing; crystalline solid (no mp taken); MS m/z (rel. int.): 404 [M] $^+$ (1.3), 386 (1.32), 358 (3), 305 (50.3), 287 (100), 245 (24), 208 (64).

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REFERENCES

- Bohlmann, F. and Zdero, C. (1979) *Chem. Ber.* **112**, 427.
- Bohlmann, F. and Zdero, C. (1979) *Chem. Ber.* **112**, 435.
- Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 855.
- Singh, P., Jakupovic, J. and Bohlmann, F. (1985) *Phytochemistry* **24**, 1525.
- Zdero, C., Bohlmann, F., King, R. M. and Robinson, H. (1986) *Phytochemistry* **25**, 2873.
- Roberts, J. S. (1981) *Terpenes. Specialist Periodical Reports* **10**, 99.

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A SESQUITERPENE LACTONE, SINTENIN, FROM *ACHILLEA SINTENISII*

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Key Word Index—*Achillea sintenisii*; Compositae; sesquiterpene lactone; costunolide; flavonoids.

Abstract—The aerial parts of *Achillea sintenisii* afforded in addition to known compounds a new costunolide, sintenin whose structure was elucidated by spectral methods.

INTRODUCTION

Achillea species have shown antibacterial [1], anti-inflammatory [2], antilarval [3], anti-allergic [4] and anti-irritant [5] activities. In this paper *Achillea sintenisii* Hub.-Mor. (Compositae) was chemically investigated, in addition to flavonoids and terpenoid compounds a new sesquiterpene lactone, sintenin was obtained.

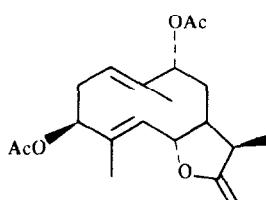
RESULTS AND DISCUSSION

The aerial parts of *A. sintenisii* afforded stigmasterol, α -amyrin, salvigenin, 6-hydroxyluteolin 6,7,3',4'-tetramethyl ether and sintenin (**1**) a new sesquiterpene lactone of the costunolide type. The IR spectrum of **1** showed a γ -lactone band at 1760 cm^{-1} , ester bands at 1720 and 1240 cm^{-1} , unsaturation at 1660 cm^{-1} . The high resolution mass spectrum gave a molecular ion peak at m/z 350 indicating a molecular formula $C_{19}H_{26}O_6$. The 1H NMR

Table 1. 1H NMR spectral data of sintenin (400 MHz, $CDCl_3$)

H	
1	5.24 br dd
3	5.11 dd
5	4.72 br d
6	4.76 dd
7	2.37 ddd
8β	2.575 ddd
9	5.17 dd
11	2.73 dq
13	1.24 s
14	1.48 s
15	1.71 s
OAc	2.03 s
OAc	2.105 s

J (Hz): $1,2\beta = 12; 1,2\alpha = 4;$
 $3\alpha,2\beta = 9; 3\alpha,2\alpha = 5.5; 5.6$
 $= 8; 6.7 = 12; 8\beta,9\beta = 10.5;$
 $9\beta,8\alpha = 5; 11.7 = 7; 11.13$
 $= 7.$



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