Pergamon

S0031-9422(96)00141-0

Phytochemistry, Vol. 42, No. 5, pp. 1399–1403, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031-9422/96 $15.00 + 0.00

SECO-PROTOLIMONOIDS FROM *TRICHILIA ELEGANS* SSP. *ELEGANS*

FERNANDA R. GARCEZ, WALMIR S. GARCEZ, EDILENE D. RODRIGUES, VALI J. POTT* and NÍDIA F. ROQUE†

Departamento de Química, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil; *Herbário CPAP, Centro de Pesquisa Agropecuária do Pantanal, Empresa Brasileira de Pesquisa Agropecuária, Corumbá, MS, Brazil; †Instituto de Química, Universidade de São Paulo, CP 26077, SP, Brazil, CEP 05599-970

(*Received in revised form* 21 *December* 1995)

Key Word Index—*Trichilia elegans* ssp. *elegans*; Meliaceae; protolimonoids.

Abstract—Three novel *seco*-A ring protolimonoids have been isolated from the seeds and bark of *Trichilia elegans* ssp. *elegans*. Their structures have been established on the basis of 1D and 2D NMR spectroscopic techniques.

INTRODUCTION

The genus *Trichilia* has been the subject of a number of investigations and is well known by the occurrence of limonoids and protolimonoids [1, 2].

Trichilia elegans ssp. *elegans* A. Juss. (Meliaceae), commonly known as 'Cachuá', is a shrub which grows in Mato Grosso do Sul, Brazil. Some plants of this genus are used in Brazilian folk medicine for the treatment of rheumatism and paludal fever, as well as emetic and purgative agents [3].

The chemistry of *T. elegans* ssp. *elegans* has not been previously studied. We describe herein the isolation and structural elucidation of three novel *seco*-A-protolimonoids (**1**–**3**) from the seeds and bark of this specimen. The structural elucidation of these isolates was mainly based on 1D and 2D NMR spectroscopic techniques.

RESULTS AND DISCUSSION

A crude ethanolic extract of the seeds was partitioned between ethanol–H_2O (1:1) and petrol. The aqueous ethanolic solution was further partitioned with dichloromethane. The dichloromethane solubles, after column chromatography on silica gel, yielded compounds **1** and **2**. Compound **3** was obtained from the dichloromethane extract of the bark, after column chromatography on silica gel and Sephadex LH-20.

The ^{1}H NMR spectrum of **1** (Table 1) displayed nine methyl singlets at: δ 0.92, 0.95, 1.16, 1.25 and 1.29 (tertiary C-methyl groups), 1.76 (vinylic), 1.95 and 1.99 (acetate methyls) and 3.63 (methoxyl).

The ^{13}C NMR spectrum (Table 2) showed signals for 35 carbon atoms and confirmed the presence of two acetates (δ 169.9, 20.9 and 170.2, 21.3). The methine carbons bearing the two acetate groups were observed at δ 74.8 and 76.6 [the ^{1}H NMR spectrum showed a broad singlet at δ 5.12 and a doublet at δ 5.47 (J = 9.8 Hz)—each), 1H—for the protons bonded to those carbons]. The presence of a trisubstituted double bond was inferred by the signals of a tertiary carbon at δ 119.4 and a quaternary carbon at δ 159.1. In the ^{1}H NMR spectrum, a doublet at δ 5.30 (J = 2.8 Hz) could be assigned to the vinylic hydrogen. The presence of a *seco*-A ring was indicated by carbon resonances at δ 171.8, 144.8 and 116.3, ascribable to C-3, C-4 and C-28, respectively. The two olefinic protons at C-28 in turn appeared as broad singlets at δ 4.82 and 5.00 and the methyl-29 was ascribed to the broad singlet at δ 1.76.

All these features, along with data obtained from homo- and hetero-nuclear chemical shift correlated 2D NMR spectra showed that **1** is closely related to nymania 2 (**4**), a 3,4-*seco*-limonoid previously isolated from *Nymania capensis* [4]. The carbon and proton shifts of the basic skeleton of **1** constituting rings A, B, C and D were very similar to those reported for **4**. However, these two compounds differ in the nature of the side chain attached at C-17. The signals due to the butyrolactone side chain present in **4** were absent in the ^{1}H NMR spectrum of **1**. Instead, two broad doublets at δ 3.42 and 3.96 (J = 10.0 Hz), a doublet at δ 2.88 (J = 9.1 Hz) and a multiplet centred at δ 3.85, indicating the presence of protons bonded to carbons bearing an oxygen function, were observed. These data, together with the remaining carbon signals displayed in the ^{13}C NMR spectrum of **1**, suggested that it was a protolimonoid with a modified eight-carbon side chain. The nature and relative positions of this side chain were apparent from cross-peak correlations observed in its ^{1}H–^{1}H COSY and ^{1}H–^{13}C HETCOR spectra. Thus, the above mentioned resonances at δ 3.42 (1H) and 3.96 (1H) were shown to be coupled in the ^{1}H–^{1}H COSY spectrum. Cross-peak correlations with the carbon resonance at δ 70.0 in the ^{1}H–^{13}C HETCOR spectrum

Table 1. ^{1}H NMR spectral data for compounds **1–3** and **3a** (200 MHz, $CDCl_3$, TMS $\delta = 0$)

H	**1***	**2**	**3**	**3a***
1	5.47 *br d* (9.8)	5.48 *br d* (10.0)	5.47 *br d* (10.4)	5.46 *br d* (10.0)
2A	2.79 *br d* (13.7)	2.80 *br d* (12.9)	2.79 *br d* (14.0)	2.78 *br d* (14.0)
2B	2.36–2.48 *m*†	2.36–2.49 *m*†	2.36–2.50 *m*†	2.30–2.47 *m*†
7	5.12 *br s*	5.14 *br s*	5.12 *br s*	5.13 *br s*
9	2.36–2.48 *m*†	2.36–2.49 *m*†	2.36–2.50 *m*†	2.30–2.47 *m*†
15	5.30 *d* (2.8)	5.29 *d* (2.9)	5.28 *d*‡ (2.7)	5.28 *d* (2.4)
Me-18	0.95 *s*	0.97 *s*	1.04 *s*‡	1.01 *s*
Me-19	0.92 *s*	0.94 *s*	0.93 *s*‡	0.86 *s*
21A	3.42 *br d* (10.0)	3.56 *br d* (10.0) H_2-21	3.56 *d* (11.0)	3.96 *dd* (12.0, 4.9)
21B	3.96 *br d* (10.0)	—	3.77–3.99 *m*§	4.26 *dd* (12.0, 2.2)
23	3.79–3.90 *m*	3.68–3.81 *m*	3.77–3.99 *m*§	5.60 *dd* (8.0, 2.2)
24	2.88 *d* (9.1)	3.42 *d* (9.2)	—	—
Me-26	1.25 *s*	1.25 *s*	1.30 *s*‡	1.38 *s*
Me-27	1.29 *s*	1.30 *s*	1.41 *s*	1.42 *s*
28A	4.82 *br s*	4.84 *br s*	4.83 *br s*	4.83 *br s*
28B	5.00 *br s*	5.01 *br s*	5.00 *br s*	5.01 *br s*
Me-29	1.76 *br s*	1.77 *br s*	1.76 *br s*	1.76 *br s*
Me-30	1.16 *s*	1.15 *s*	1.12 *s*‡	1.14 *s*
OAc	1.95 *s*, 1.99 *s*	1.96 *s*, 2.01 *s*	1.95 *s*, 2.02 *s*	1.97 *s*, 2.02 *s* 2.07 *s*, 2.07 *s*
OMe	3.63 *s*	3.65 *s*	3.65 *s*	3.65 *s*

Coupling constants (J in Hz) are given in parentheses.
*Assignments were confirmed by the ^{1}H–^{1}H COSY spectra.
†,§Overlapped signals.
‡Duplicated signals.

indicated geminal coupling. Mutual couplings between protons at δ 3.85 (1H) and 2.88 (1H) were also observed. The coupling constant of the latter (9.1 Hz) suggested their *trans*-diaxial relationship. From the ^{1}H–^{13}C HETCOR spectrum it was seen that the proton multiplet at δ 3.85 showed connectivity to the carbon at δ 64.4 and the proton doublet at δ 2.88 to the carbon at δ 86.5. The two deshielded methyl singlets at δ 1.25 and 1.29 showed cross-peak correlations with the carbon signals at δ 24.0 and 28.5, respectively. They were attached to a quaternary carbon bearing an oxygen atom; a signal at δ 74.2 in the ^{13}C NMR spectrum could be assigned to this carbon. The signals observed at δ 35.9 (CH) and 36.3 (CH_2) accounted for the remaining carbons of the side chain at C-17 in **1**.

The data given clearly suggest that this side chain is the same as that found in bourjotinolone A, the structure of which has been well established [5–7]. The close similarity between the chemical shifts and coupling constants of the C-21, C-23 and C-24 protons in bourjotinolone A and **1** indicates that they have the same stereochemistry in the side chain. The structure of this protolimonoid was thus established as **1**.

Comparison of the spectral properties [^{1}H (Table 1) and ^{13}C NMR (Table 2)] of **1** with those of **2** suggested they were closely related except for the presence in the latter of a C-21, C-25 seven-membered oxide ring with two hydroxyl groups at C-23 and C-24. The resonance of C-24 was shifted upfield from **1** to **2** ($\Delta\delta$ 5.8) by the loss of a β-effect. The same occurred with that of C-21 ($\Delta\delta$ 5.8), which has two methyl groups at the γ-position, unlike C-21 in **1**. Otherwise, C-23 is deshielded in **2** ($\Delta\delta$ 3.6) because of the lack of an isopropyl group γ-effect.

The side chain of **2** was identical with that found in some protolimonoids, e.g. sapelin B, the stereochemistry of which has been defined [8, 9]. The assignments of the ^{1}H and ^{13}C NMR spectra of **2** compare well with those reported for this side chain [6, 7]. Thus, structure **2** is as assigned.

The ^{1}H and ^{13}C NMR spectra of **3** were similar in many respects to those of **1**. The carbon and proton shifts of the rings A, B, C and D corresponded to each other, so that these rings should be identical. However, some interesting features appeared with respect to the nature of the side chain in **3**.

The ^{1}H NMR spectrum of **3** on comparison with that of **1** (Table 1) showed a downfield shift of the H-23

Table 2. ^{13}C NMR spectral data for compounds **1–3** and **3a** (50 MHz, $CDCl_3$, TMS $\delta = 0$)

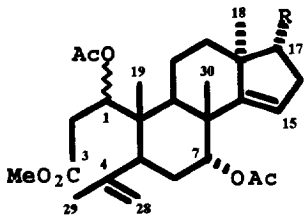
C	1*	2	3	3a
1	76.6 *d*	76.6 *d*	76.6 *d*	76.4 *d*
2	35.3 *t*	35.3 *t*	35.3 *t*	35.2 *t*
3	171.8 *s*	171.8 *s*	171.8 *s*	171.6 *s*
4	144.8 *s*	144.8 *s*	144.9 *s*	144.7 *s*
5	34.6 *d*	34.4 *d*	34.4 *d*	34.4 *d*
6	35.0 *t*	34.9 *t*	34.8 *t*†	34.7 *t*
7	74.8 *d*	74.6 *d*	74.6 *d*†	74.5 *d*
8	42.3 *s*	42.3 *s*	42.2 *s*†	42.3 *s*
9	44.1 *d*	44.1 *d*	44.0 *d*	44.0 *d*
10	44.0 *s*	44.0 *s*	44.0 *s*†	44.0 *s*
11	18.5 *t*	18.5 *t*	18.4 *t*†	18.4 *t*
12	29.2 *t*	29.0 *t*	29.1 *t*	29.1 *t*
13	46.0 *s*	45.9 *s*	46.2 *s*	46.2 *s*
14	159.1 *s*	159.3 *s*	158.9 *s*	159.2 *s*
15	119.4 *d*	118.9 *d*	119.0 *d*†	118.9 *d*
16	34.8 *t*	34.2 *t*	33.8 *t*†	34.1 *t*
17	52.4 *d*	54.2 *d*	57.2 *d*	55.3 *d*
18	20.2 *q*	19.9 *q*	19.8 *q*	19.4 *q*
19	15.0 *q*	15.0 *q*	15.0 *q*	15.0 *q*
20	35.9 *d*	36.4 *d*	35.5 *d*	36.7 *d*
21	70.0 *t*	64.2 *t*	65.3 *t*†	65.5 *t*
22	36.3 *t*	37.9 *t*	32.8 *t*†	31.2 *t*
23	64.4 *d*	68.0 *d*	67.4 *d*†	73.6 *d*
24	86.5 *d*	80.7 *d*	95.9 *s*†	211.4 *s*
25	74.2 *s*	76.1 *s*	77.6 *s*	77.4 *s*
26	24.0 *q*	22.4 *q*	24.2 *q*‡	27.7 *q*‡
27	28.5 *q*	26.1 *q*	24.5 *q*‡	27.4 *q*‡
28	116.3 *t*	116.3 *t*	116.3 *t*	116.4 *t*
29	22.7 *q*	22.7 *q*	22.8 *q*†	22.8 *q*
30	26.7 *q*	26.7 *q*	26.7 *q*†	26.7 *q*
OAc	169.9 *s*; 170.2 *s*	170.0 *s*; 170.3 *s*	170.0 *s*; 170.2 *s*	169.9 *s*; 170.3 *s*
	20.9 *q*; 21.3 *q*	20.9 *q*; 21.3 *q*	21.0 *q*; 21.3 *q*	170.8 *s*; 171.2 *s*
				20.6 *q*; 21.0 *q*
				21.0 *q*; 21.3 *q*
OMe	52.0 *q*	52.0 *q*	52.0 *q*†	52.0 *q*

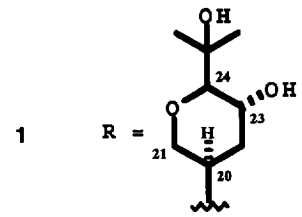
Multiplicities were established from the DEPT pulse sequence.
*Assignments were confirmed by the ^1H–^{13}C HETCOR spectrum.
†Duplicated signals.
‡Assignments interchangeable.

multiplet and lacked the doublet attributed to H-24 in **1**. The ^{13}C NMR spectrum showed double signals, particularly those belonging to the side chain at C-17 (Table 2). The presence of a quaternary carbon resonance occurring as double peaks at δ 95.9/97.5 indicated a hemiacetal carbon and was attributed to C-24. The two epimeric forms at C-24 could account for the multiple nature of the ^1H and ^{13}C NMR spectra. Resonances of C-23 and C-25 were shifted downfield from **1** to **3** ($\Delta\delta$ 3.0 and 3.4, respectively) by the hydroxyl group at C-24, and C-21 and C-22 moved upfield ($\Delta\delta$ 4.7 and 3.5, respectively) on the introduction of a *γ-gauche*-hydroxyl group.

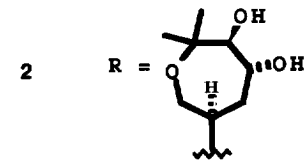
Acetylation of **3** (acetic anhydride–pyridine, overnight) yielded **3a**, whose ^1H NMR spectrum (Table 1) showed the presence of two new acetate groups and a downfield shift of H_2-21, which now appeared as two double doublets at δ 3.96 (J = 12.0 and 4.9 Hz) and 4.26 (J = 12.0 and 2.2 Hz), and of H-23, as a double doublet at δ 5.60 (J = 8.0 and 2.2 Hz). In the ^1H–^1H COSY NMR spectrum, mutual couplings between H-21a and H-21b, H_2-21 and H-20 and between H-23 and H_2-22 were observed. The ^{13}C NMR spectrum (Table 2) lacked the hemiacetal carbon resonances at δ 95.9/97.5 and displayed instead a carbonyl signal at δ 211.4. A downfield shift for C-23 to δ 73.6 was also observed. These data were in accordance with structure **3a** formed by the acetylation of the opened side chain of **3**. The above information could be satisfactorily assembled to give structure **3** for this protolimonoid.

The hemiacetal side chain at C-17 depicted in **3** is not common and was first reported in spicatin, a protolimonoid isolated from *Entandrophragma spicatum* [10], and, recently, in meliavolin (**5**), from *Melia volkensii* [11]. The stereochemistry of this side chain was well established in the latter as being 20*S*,

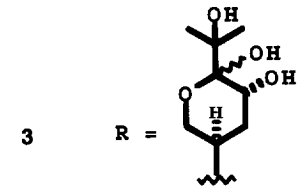




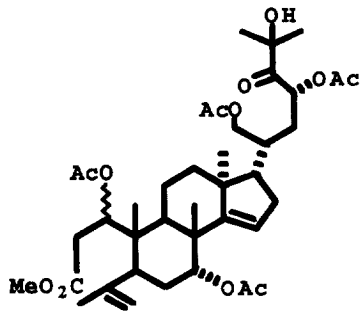
1



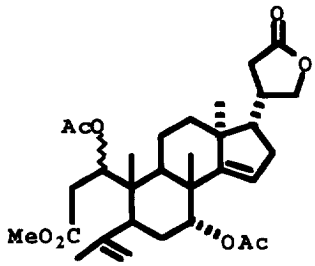
2



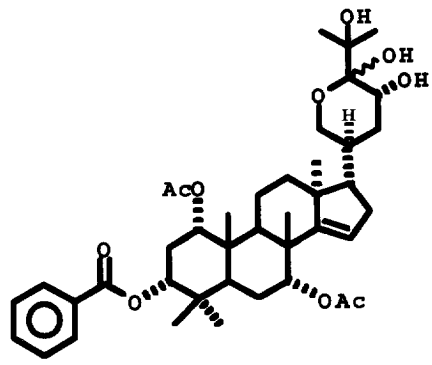
3



3a



4



5

23*R*. The ^{1}H and ^{13}C NMR data reported for this side chain [11] showed a close resemblance to those for **3**, as well as its keto diacetate derivative to **3a**. On the basis of these data, the side chain at C-17 in **3** is the same as in meliavolin.

Compound **1** afforded a molecular ion peak at *m/z*

618 in the EI mass spectrum. In the spectra of **2** and **3**, the peak of highest mass, *m/z* 600 and 616, respectively, was attributed to $[M - H_2O]^+$ in each instance.

EXPERIMENTAL

General. EIMS: direct inlet with 70 eV ionization. ^1H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded with TMS as int. standard. Silica gel 60 was used for chromatography: 70–230 mesh for CC and 230–400 mesh for flash CC. Sephadex LH-20 was used for molecular exclusion chromatography.

Plant material. The seeds and bark of *T. elegans* ssp. *elegans*. A. Juss. were collected in Corumbá, Mato Grosso do Sul, Brazil, in February 1990 and August 1990, respectively. The plant was identified by Prof. Humberto Barreiros, of the Jardim Botânico do Rio de Janeiro, Brazil, where voucher specimens are deposited.

Extraction and isolation of compounds. Air dried and ground seeds of *T. elegans* ssp. *elegans* (225 g) were extracted at room temp. with hexane and EtOH, successively. The EtOH extract, after concn *in vacuo*, was partitioned between EtOH–H_2O (1:1) and petrol. The aq. EtOH phase was extracted with CH_2Cl_2. The CH_2Cl_2 phase was subjected to CC on silica gel, eluted with increasing amounts of EtOAc in hexane and EtOAc–MeOH (9:1). Flash CC of the fr. eluted with EtOAc (CH_2Cl_2–EtOAc and MeOH gradient as solvent) yielded **1** (22 mg—0.01% yield) and **2** (16 mg—0.007% yield).

Methyl - 1ξ,7R - *diacetoxy* - 23R,25 - *dihydroxy* - 20S,24R - 21,24 - *epoxy* - 3,4 - seco - *apotirucall* - 4(28),14(15)-*diene*-3-*oate* (**1**). Obtained as a gum. EIMS *m/z* (rel. int.): 618 $[M]^{+\cdot}$ (0.5), 600 $[M - H_2O]^{+\cdot}$ (2.5), 582 $[M - 2H_2O]^{+\cdot}$ (1.9), 253 (21), 131 (100), 103 (40), 59 (50). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1742, 1241, 1173, 1031. ^1H and ^{13}C NMR: Tables 1 and 2, respectively.

Methyl-1ξ,7R-*diacetoxy*-23R,25S-*dihydroxy*-20S-21,25-*epoxy*-3,4-seco-*apotirucall*-4(28),14(15)-*diene*-3-*oate* (**2**). Obtained as a gum. EIMS *m/z* (rel. int.): 600 $[M - H_2O]^{+\cdot}$ (0.2), 582 $[M - 2H_2O]^{+\cdot}$ (0.4), 149 (46), 131 (17), 84 (28), 57 (100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3457, 1740, 1241, 1171, 1031. ^1H and ^{13}C NMR: Tables 1 and 2, respectively.

Air dried and ground bark (1 kg) of *T. elegans* ssp. *elegans* was successively extracted at room temp. with hexane, CH_2Cl_2 and EtOH. The CH_2Cl_2 extract was subjected to CC on silica gel, eluted with a hexane–Me_2CO gradient and Me_2CO–MeOH (9:1). The fr. eluted with hexane–Me_2CO (7:3), after CC on Sephadex LH-20, sequentially eluted with hexane–CH_2Cl_2 (1:4), CH_2Cl_2–Me_2CO (3:2) and (1:4), yielded **3** (15 mg—0.002% yield) as a gum.

Methyl-1ξ,7R-*diacetoxy*-23R,24,25-*trihydroxy*-20S-21,24-*epoxy*-3,4-seco-*apotirucall*-4(28),14(15)-*diene*-3-*oate* (**3**). EIMS *m/z* (rel. int.): 616 $[M - H_2O]^{+\cdot}$ (0.2), 598 $[M - 2H_2O]^{+\cdot}$ (0.5), 149 (24), 131 (17), 84 (25), 55 (100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3433, 1736, 1241, 1175, 1032. ^1H and ^{13}C NMR: Tables 1 and 2, respectively. Acetylation of **3** (11 mg) in Ac_2O (0.9 ml) and pyridine (0.9 ml) overnight at room temp., followed by normal work-up, gave **3a** (9 mg), as a gum.

Methyl-1ξ,7R,21,23R-*tetra*-*acetoxy*-24-*one*-25-*hydroxy*-20S-3,4-seco-*apotirucall*-4(28),14(15)-*diene*-3-*oate* (**3a**). ^1H and ^{13}C NMR: Tables 1 and 2, respectively.

Acknowledgements—The authors are grateful to CPq-PROPP/UFMS, CECITEC-MS and FAPESP for financial support and to CAPES-PICDT (F. R. G.) and CNPq (N. F. R.) for the award of a scholarship.

REFERENCES

1. Taylor, D. A. H. (1984) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W. eds), Vol. 45, p. 1. Springer, New York.
2. Banerji, B. and Nigam, S. K. (1984) *Fitoterapia* **LV**, 3.
3. Corrêa, M. P. (1984) *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*. Ministério da Agricultura, Rio de Janeiro.
4. MacLachlan, L. K. and Taylor, D. A. H. (1982) *Phytochemistry* **21**, 1701.
5. Breen, G. J. W., Ritchie, E., Sidwell, W. T. L. and Taylor, W. C. (1966) *Aust. J. Chem.* **19**, 455.
6. Jolad, S. D., Hoffmann, J. J., Cole, J. R., Tempesta, M. S. and Bates, R. B. (1980) *J. Org. Chem.* **45**, 3132.
7. Kishi, K., Yoshikawa, K. and Arihara, S. (1992) *Phytochemistry* **31**, 1335.
8. Chan, W. R., Taylor, D. R. and Yee, T. (1970) *J. Chem. Soc. (C)* 311.
9. Lyons, C. W. and Taylor, D. R. (1976) *J. Chem. Soc., Chem. Commun.* 647.
10. Connolly, J. D., Phillips, W. R., Mulholland, D. A. and Taylor, D. A. H. (1981) *Phytochemistry* **20**, 2596.
11. Zeng, L., Gu, Z., Fang, X., Fanwick, P. E., Chang, C., Smith, D. L. and McLaughlin, J. L. (1995) *Tetrahedron* **51**, 2477.