PERGAMON

Phytochemistry 51 (1999) 931–935

PHYTOCHEMISTRY

A furostanol glycoside from rhizomes of *Costus spicatus*

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Received 30 June 1998; accepted 28 September 1998

Abstract

A new furostanol glycoside was isolated from the rhizomes of *Costus spicatus*. Its structure was established as (3β,22α,25*R*)-26-(β-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl *O*-D-apio-β-D-furanosyl-(1 → 2)-*O*-[6-deoxy-α-L-mannopyranosyl-(1 → 4)]-β-D-glucopyranoside. The structural identification was performed using detailed analysis of ^{1}H and ^{13}C NMR spectra including 2D NMR spectroscopic techniques (COSY, HETCOR and COLOC) and chemical conversions.

Keywords: Costus spicatus; Costaceae; Rhizomes; Steroidal saponin; Furostanol glycoside

1. Introduction

Costus spicatus Swartz (Syn.: *C. cylindricus* Jacq.), commonly called 'cana do brejo' in Brazil, is a native species found in wet coastal forests from southern Mexico, Yucatan and Costa Rica to northern Colombia and Brazil. Peasants of the interior of Brazil often drink the plant juice with added water and sugar as a cooling beverage in hot weather (Cruz, 1965). The rhizome of this plant is boiled and the purplish tea taken as a diuretic to relieve complaints of the bladder and urethra and to expel kidney stones (Manfred, 1947). Several *Costus* species have attracted attention as a new source of diosgenin, a precursor of steroidal hormones (Mahato, Ganguly, & Sahu, 1982; Dixit, Srivastava, Dixit, & Chandell, 1991; Chen & Yin, 1995; Inoue, Kobayashi, Nogushi, Sankawa, & Ebizuka, 1995; Lin, Hanquet, & Lacaille-Dubois, 1996; Lin, Lacaille-Dubois, Hanquet, Correia, & Chauffert, 1997). Nonetheless, no chemical studies have been carried out on the constituents of *C. spicatus*. As part of our ongoing investigation for biologically active saponins (Bernardo, Pinto, & Parente, 1996; Daros, Matos, & Parente, 1996; Pereira, Daros, Parente, & Matos, 1996a, 1996b), we report in this paper the isolation and structural elucidation of a new saponin **1**.

2. Results and discussion

The rhizomes of *C. spicatus* were extracted with methanol. After concentration under reduced pressure, the methanol extract was partitioned between water and *n*-butanol. Chromatographic separations of the organic phase on Sephadex LH-20 and silica gel gave compound **1** which was detected with orcinol–H_2SO_4 reagent. Compound **1** was obtained as colourless needles and gave a positive Liebermann–Burchard test for a steroidal saponin. The LSIMS showed an ion peak $[M–H]^-$ at *m/z* 1047 which, together with ^{13}C spectral data (Tables 1 and 2), suggested the molecular formula as $C_{51}H_{84}O_{22}$. Other significant peaks visible at *m/z* 915 [(M–H)-132], 901 [(M–H)-146] and 885 [(M–H)-162] correspond to the loss of a terminal pentose, a terminal deoxyhexose and a terminal hexose, respectively.

In addition to this, the furostanol glycosidic nature of **1** was indicated by the strong absorption bands at 3430 and 1050 cm^{-1} and a 25*R*-furostan steroidal structure (813, 838 and 913 cm^{-1}, intensity 913 < 838 cm^{-1}) in the IR spectrum (Wall, Eddy, McClennan, &

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00077-1

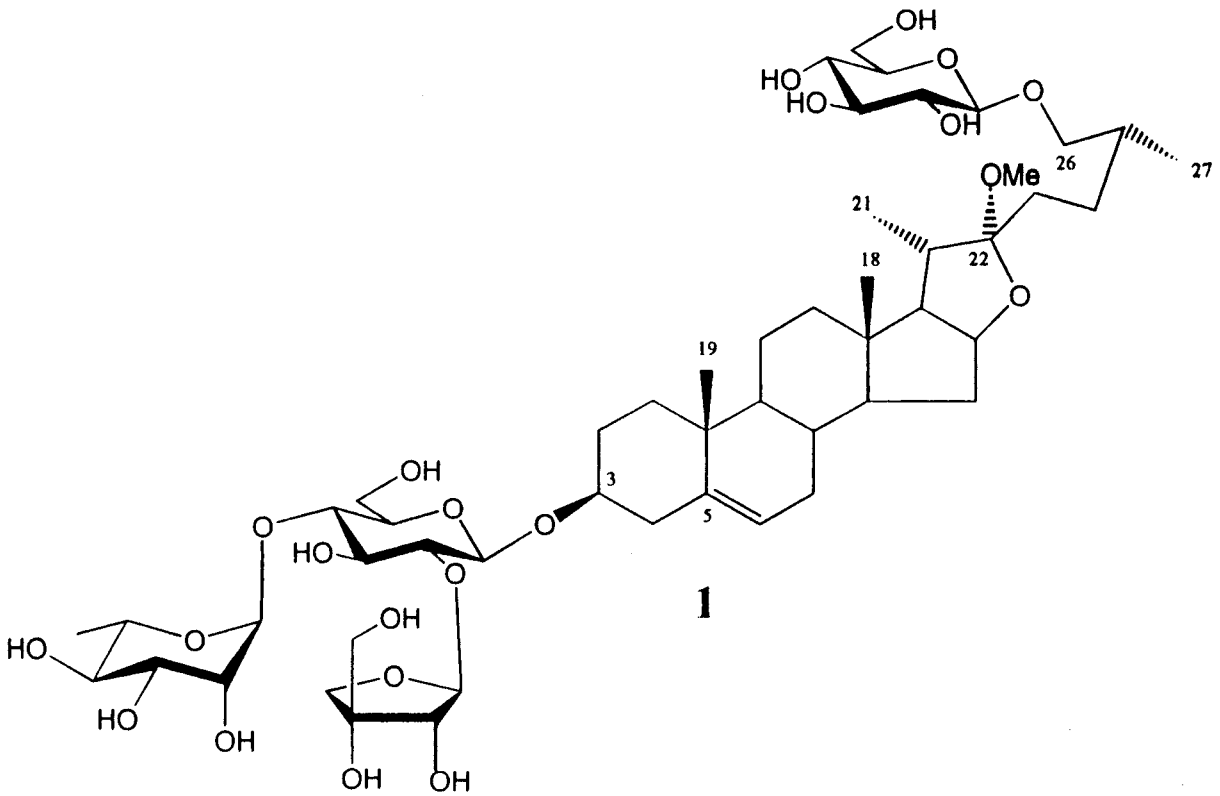

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Table 1
^{13}C NMR spectral data ofr the aglycone moieties of **1** and **1a** in pyridine-d_5

C	1	1a	DEPT
1	37.0	37.0	CH_2
2	29.7	29.5	CH_2
3	77.9	77.9	CH
4	38.5	38.5	CH_2
5	140.3	140.3	C
6	121.4	121.4	CH
7	32.0	32.0	CH_2
8	31.2	31.2	CH
9	49.9	49.9	CH
10	36.7	36.7	C
11	20.7	20.7	CH_2
12	39.5	39.3	CH_2
13	40.3	40.5	C
14	56.1	56.1	CH
15	31.9	31.9	CH_2
16	80.8	80.6	CH
17	63.7	63.5	CH
18	16.0	16.0	CH_3
19	18.9	18.9	CH_3
20	40.2	40.1	CH
21	16.6	16.8	CH_3
22	112.2	112.6	C
23	30.3	30.5	CH_2
24	27.9	27.7	CH_2
25	33.8	33.8	CH
26	74.7	74.5	CH_2
27	16.9	16.9	CH_3
22-OMe	46.8	46.8	CH_3

Klumpp, 1952), confirmed by ^1H and ^{13}C NMR spectra (Tables 1 and 2 Table 3) (Ju & Jia, 1992; Mimaki, Sashida, Nakamura, Nikaido, & Ohmoto, 1993; Lin et al., 1996Lin et al., 1997; Hu, Dong, Yao, Kobayashi, & Iwasaki, 1997; Kawano, Sato, & Sakamura, 1997; Shao et al., 1997). The ^1H-NMR spectral data Table 3 contained signals for an olefinic proton at δ 5.30 (br s), four anomeric protons at δ 6.28 (br s), 5.95 (d, $J=3.6$ Hz), 4.98 (d, $J=7.7$ Hz) and 4.82 (d, $J=7.8$ Hz), methoxyl protons at δ 3.28 (s), three secondary methyl protons at δ 1.78 (d, $J=6.3$ Hz), 1.20 (d, $J=6.8$ Hz) and 0.98 (d, 6.6 Hz) and two angular methyl protons at δ 1.06 and 0.84 (each s). The signal at δ 1.78 was due to the methyl group of rhamnose.

The above ^1H NMR spectral data and a comparison of the ^{13}C NMR signals of the aglycone moiety of **1** Table 1 with those described in the literature [14,16] showed the structure of the aglycone to be (3β,22α,25*R*)-22-methoxyfurost-5-en-3,26-diol. The α-configuration of C-22 methoxy group of the aglycone moiety was defined by a rotating-frame Overhauser effect (ROE) study. In the ^{13}C NMR spectrum of **1**, a 2,4-linked inner β-D-glucopyranosyl unit, a terminal β-D-glucopyranosyl unit, a terminal α-L-rhamnopyranosyl unit and a terminal β-D-apiofuranosyl unit were clearly observed. In addition to this, the COLOC spectrum displayed long range couplings between inner glucose-H-1 at δ 4.98 and aglycone-C-3 at δ 4.82 and

Table 2
^{13}C NMR spectral data for the carbohydrate moieties of **1** and **1a** in pyridine-d_5[a]

C	DEPT	1	1a	H-C LR
iGlc				
1	CH	99.8	100.3	Agl[b]-C-3
2	CH	77.4	75.2	
3	CH	77.7	76.8	
4	CH	78.9	79.6	
5	CH	76.9	76.9	
6	CH_2	60.8	61.6	
tGlc				
1	CH	104.5	105.1	Agl-C-26
2	CH	74.7	75.3	
3	CH	78.1	78.6	
4	CH	71.9	71.8	
5	CH	78.9	78.1	
6	CH_2	62.3	62.9	
Rha				
1	CH	102.4	102.9	iGlc-C-4
2	CH	72.2	72.6	
3	CH	72.3	72.9	
4	CH	73.6	74.2	
5	CH	69.9	70.5	
6	CH_3	18.2	18.8	
Api				
1	CH	110.7		iGlc-C-2
2	CH	77.2		
3	C	79.6		
4	CH_2	74.8		
5	CH_2	64.2		

[a] The assignments were made on the basis of DEPT and COLOC experiments.
[b] Agl = aglycone.

Table 3
Selected characteristic ^1H NMR spectral data [δ (ppm), J (Hz)] for **1** and **1a** in pyridine-d_5. The following conventions is used: iGlc = inner glucose, tGlc = terminal glucose

	1	1a	COSY (^1H)
H-6	5.30 br s	5.30 br s	H-7
18-Me	0.84 s	0.83 s	
19-Me	1.06 s	1.06 s	
21-Me	1.20 d (6.8)	1.21 d (6.8)	H-20
27-Me	0.98 d (6.6)	0.98 d (6.6)	H-25
22-OMe	3.28 s	3.27 s	
Sugar methyl group and anomeric protons			
Rha–Me	1.78 d (6.3)	1.79 d (6.2)	Rha-H-5
iGlc-H-1	4.98 d (7.7)	4.93 d (7.8)	iGlc-H-2
tGlc-H-1	4.82 d (7.8)	4.84 d (7.7)	tGlc-H-2
Rha-H-1	6.28 br s	6.40 br s	Rha-H-2
Api-H-1	5.95 d (3.6)		Api-H-2

aglycone-C-26 at δ 74.7, between apiose-H-1 at δ 5.95 and glucose-C-2 at δ 77.4 and between rhamnose-H-1 at δ 6.28 and glucose-C-4 at δ 78.9, indicating that the apiose and rhamnose were linked to the C-2 and C-4 of the inner glucose, respectively. These results were confirmed by comparison with those reported in the literature (Lin et al., 1996, 1997) and by methylation analysis (Fournet, Dhalluin, Leroy, Montreuil, & Mayer, 1978; Fournet, Strecker, Leroy, & Montreuil, 1981; Parente et al., 1985).

On acid hydrolysis, **1** gave a pseudosapogenin, glucose, rhamnose and apiose. The pseudosapogenin was identified as diosgenin by direct comparison of TLC, m.p., IR, ^1H and ^{13}C NMR and EIMS with an authentic sample. The molar carbohydrate composition of **1** indicated the presence of four neutral monosaccharides: glucose:rhamnose:apiose (2.0:0.9:0.6) (the molar responses of rhamnose and apiose are taken as 1.0) (Kamerling, Gerwig, Vliegenthart, & Clamp, 1975). Their absolute configurations were determined by GC of their TMSi (−)-2-butylglycosides (Gerwig, Kamerling, & Vliegenthart, 1978). D-glucose, L-rhamnose and D-apiose were detected. Compound **1** was submitted to partial hydrolysis with 0.2 M hydrochloric acid in methanol to yield the prosapogenin (3β,22α,25*R*)-26-(β-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl *O*-6-deoxy-α-L-rhamno-pyranosyl-(1 → 4)-β-D-glucopyranoside (**1a**) which was elucidated by spectroscopic techniques and chemical conversions. Its carbohydrate composition was established as glucose and rhamnose in the molar ratio 2:1. The methylation analysis of **1a** showed a 4-linked glucose, a terminal glucose and a terminal rhamnose. This was direct evidence that in **1** the apiose residue was linked at C-2 position of the inner glucose. Consequently, on the basis of IR, ^1H and ^{13}C NMR spectroscopy, LSIMS and chemical reactions, the structure of **1** was established as (3β,22α,25*R*)-26-(β-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl *O*-D-apio-β-D-furanosyl-(1 → 2)-*O*-[6-deoxy-α-L-mannopyranosyl-(1 → 4)]-β-D-glucopyranoside.

3. Experimental

3.1. General

M.p.'s were determined by a Ellectrothermal 9200 micro-melting point apparatus and are uncorr. OR were measured on a Perkin Elmer 243B polarimeter. IR spectra were measured on a Perkin Elmer 599B. ^1H and ^{13}C NMR spectra were obtained on a Bruker AMX-400 NMR spectrometer. ^1H NMR: 400 MHz, in pyridine-d_5. TMS as int. standard. ^{13}C NMR edited DEPT spectra: 100 MHz from pyridine-d_5 solns. GC was carried out with FID, using a capillary column

(0.3 mm × 25 m) OV 101. LSIMS, EIMS and GC–MS were taken on a VG Auto SpecQ spectrometer. EIMS and GC–MS: recorded at 70 eV. Negative LSIMS carried out using thioglycerol as the matrix and Cs ions accelerated at 35 kV. Acceleration voltage: 8 kV. Silica gel columns (230–400 mesh ASTM, Merck) and Sephadex LH-20 (Pharmacia) were used for CC. TLC was performed on silica gel coated plates (Merck) using the following solvent systems: (A) $CHCl_3$–MeOH–H_2O (65:35:10) for furostanol glycosides **1** and **1a**, (B) toluene–Me_2CO (4:1) for sapogenin and (C) *n*-BuOH–pyridine–H_2O (6:4:3) for monosaccharides. Spray reagents were orcinol–H_2SO_4 for furostanol glycosides **1** and **1a**, $CeSO_4$ for sapogenin and aniline–diphenylamine–85% orthophosphoric acid–MeOH (1:1:5:43) for monosaccharides.

3.2. Plant material

The rhizomes of *C. spicatus* Swartz, were collected at Ilha do Fundão, Rio de Janeiro, in September 1996 and identified by Luci Senna Valle. A voucher specimen (No. R192950) is deposited at the herbarium of the National Museum, Rio de Janeiro, Brazil.

3.3. Extraction and isolation

Dried powdered rhizomes (700 g) were extracted with MeOH (7 l). The combined MeOH solns were concd in vacuo to give 5 g dry extract. A suspension of the resulting extract in H_2O was partitioned successively with *n*-BuOH to afford 2 g residue. It was roughly chromatographed on Sephadex LH-20 with MeOH to give 1 g steroidal glycoside mixt. This was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (70:30:10) to yield one TLC homogeneous compound **1** (320 mg), R_f 0.37 which gave a dark green colour with orcinol–H_2SO_4.

3.4. Compound *1*

Colourless needles from MeOH, m.p. 222–224°C, $[\alpha]_D^{20}$ −102° (MeOH, *c* 0.001). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1050 (C–O), 913, 838, 813, 638 [(25*R*)-furostanol, intensity 913 < 838]. Negative LSIMS, *m/z*: 1047 $[M–H]^-$, 915 $[(M–H)]\text{-}132]^-$, 901 $[(M–H)\text{-}146]^-$, 885 $[(M–H)]\text{-}162]^-$. ^1H and ^{13}C NMR spectral data shown in Tables 1–3.

3.5. Acid hydrolysis of *1*

Compound **1** (50 mg) in 1 M HCl (H_2O–dioxane, 1:1) (20 ml) was heated in a sealed tube for 1 h at 100°C. After cooling, the reaction mixt. was neutralized with 3% KOH MeOH and evapd to dryness. The salts that deposited on addition of MeOH were filtered off and the filtrate was passed through a Sephadex LH-20 with MeOH to give the hydrolysate (47 mg) which was chromatographed on silica gel CC with $CHCl_3$–MeOH–H_2O (7:3:0.2) to yield the diosgenin (15 mg) and a sugar mixt. Identity of diosgenin was established by comparison with an authentic sample through m.p., IR, ^1H and ^{13}C NMR and EIMS. The sugar mixt. was dissolved in pyridine and analyzed by silica gel-TLC in the above described solvent system. After spraying, apiose gave a weak yellow spot at R_f 0.78, rhamnose gave a green spot at R_f 0.75 and glucose gave a blue spot at R_f 0.70. For co-chromatography the hydrolysate of bredemeyeroside B (Daros et al., 1996) was used.

3.6. Partial hydrolysis of *1*

Compound **1** (100 mg) in 0.2 M HCl in MeOH (5 ml) was refluxed for 35 min. The reaction mixt. was neutralized by the procedure described above. The hydrolysate was chromatographed on Sephadex LH-20 with MeOH to give the prosapogenin **1a** (73 mg), R_f 0.58 (silica gel-TLC, solvent system A) which gave a dark green colour with orcinol–H_2SO_4 and a sugar residue which was dissolved in pyridine, analyzed by silica gel-TLC as described above and apiose was detected.

3.7. Compound *1a*

Colourless needles from MeOH, m.p. 186–188°C, $[\alpha]_D^{20}$ −78° (MeOH, *c* 0.001). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1052 (C–O), 913, 838 [(25*R*)-furostanol, intensity 913 < 838]. Negative LSIMS, *m/z*: 915 $[M–H]^-$, 769$[(M–H)\text{-}146]^-$, 753 $[(M–H)\text{-}162]^-$. ^1H and ^{13}C NMR spectral data shown in Tables 1–3.

3.8. Molar carbohydrate composition and D, L configurations

The molar carbohydrate compositions of **1** and **1a** were determined by GC–MS analyses of their monosaccharides as their TMSi methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 h, 80°C) and trimethylsilylation according to Kamerling et al. (1975). The configurations of the glycosides were established by capillary GC of their TMSi (−)-2-butylglycosides (Gerwig et al., 1978).

3.9. Methylation analysis

Compounds **1** and **1a** were methylated with DMSO-lithium methylsulphinyl carbanion-CH_3I (Parente et al., 1985). The methyl ethers were obtained either (a) after hydrolysis (4 M TFA, 100°C) and analyzed as partially polyol-acetates by GC–MS (Fournet et al.,

1978) or (b) after methanolysis (0.5 M HCl in MeOH, 24 h, 80°C) and analyzed as partially methylated methylglycosides by GC–MS (Fournet et al., 1981).

Acknowledgements

The authors are grateful to Eduardo M.B. da Silva, Maria C.P. Lima and Alexandre F. Neves for their contribution in the acquisition of the spectra. Financial support from CNPq and PADCT are also acknowledged.

References

Bernardo, R. R., Pinto, A. V., & Parente, J. P. (1996). *Phytochemistry*, *43*, 465.

Chen, C., & Yin, H. (1995). *Tianran Chanwu Yanjiu Yu Kaifa*, *7*, 18.

Cruz, G. L. (1965). In *Livro Verde das Plantas Medicinais e Industriais do Brasil* (p. 864). Belo Horizonte, Brazil: Velloso S.A.

Daros, M. R., Matos, F. J. A., & Parente, J. P. (1996). *Planta Medica*, *62*, 523.

Dixit, B. S., Srivastava, S. N., Dixit, G. S., & Chandell, P. S. (1991). *Indian Journal of Pharmaceutical Sciences*, *53*, 251.

Fournet, B., Dhalluin, J. M., Leroy, Y., Montreuil, J., & Mayer, H. (1978). *Journal of Chromatography*, *153*, 91.

Fournet, B., Strecker, G., Leroy, Y., & Montreuil, J. (1981). *Analytical Biochemistry*, *116*, 489.

Gerwig, G. J., Kamerling, J. P., & Vliegenthart, J. F. G. (1978). *Carbohydrate Research*, *62*, 349.

Hu, K., Dong, A. J., Yao, X. S., Kobayashi, H., & Iwasaki, S. (1997). *Phytochemistry*, *44*, 1339.

Inoue, K., Kobayashi, S., Nogushi, H., Sankawa, U., & Ebizuka, Y. (1995). *Natural Medicine*, *49*, 336.

Ju, Y., & Jia, Z. J. (1992). *Phytochemistry*, *31*, 1349.

Kamerling, J. P., Gerwig, G. J., Vliegenthart, J. F. G., & Clamp, J. R. (1975). *Biochemical Journal*, *151*, 491.

Kawano, K., Sato, H., & Sakamura, S. (1997). *Agricultural and Biological Chemistry*, *41*, 1.

Lin, R. C., Hanquet, B., & Lacaille-Dubois, M. A. (1996). *Phytochemistry*, *43*, 665.

Lin, R. C., Lacaille-Dubois, M. A., Hanquet, B., Correia, M., & Chauffert, B. (1997). *Journal of Natural Products*, *60*, 1165.

Mahato, S. B., Ganguly, A. N., & Sahu, N. P. (1982). *Phytochemistry*, *21*, 959.

Manfred, L. (1947). In *7,000 Recetas Botânicas a Base de 1,300 Plantas Medicinales Americanas* (p. 778). Buenos Aires, Argentina: Editorial Kier.

Mimaki, Y., Sashida, Y., Nakamura, O., Nikaido, T., & Ohmoto, T. (1993). *Phytochemistry*, *33*, 675.

Parente, J. P., Cardon, P., Leroy, Y., Montreuil, J., Fournet, B., & Ricart, G. (1985). *Carbohydrate Research*, *141*, 41.

Pereira, B. M. R., Daros, M. R., Parente, J. P., & Matos, F. J. A. (1996a). *Fitoterapia*, *67*, 323.

Pereira, B. M. R., Daros, M. R., Parente, J. P., & Matos, F. J. A. (1996b). *Phytotherapy Research*, *10*, 666.

Shao, Y., Poobrasert, O., Kennelly, E. J., Chin, C. K., Ho, C. T., Huang, M. T., Garrison, S. A., & Cordell, G. A. (1997). *Planta Medica*, *63*, 258.

Wall, M. E., Eddy, C. R., McClennan, M. L., & Klumpp, M. E. (1952). *Analytical Chemistry*, *24*, 1337.