Epi-Sarsasapogenin and *epi*-smilagenin: two sapogenins isolated from the rumen content of sheep intoxicated by *Brachiaria decumbens*

Nordin H. Lajis, A. Salam H. Abdullah,* S. Jalaludin S. Salim,* John B. Bremner† and Mohammad Niyaz Khan

*Department of Chemistry, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor D. E., Malaysia. *Department of Animal Sciences, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor D. E., Malaysia. †Department of Chemistry, University of Wollongong, PO Box 1144, Wollongong, NSW 2500, Australia.*

Spectroscopic examination of purified extracts of the rumen content of sheep intoxicated by Brachiaria decumbens *revealed the presence of two spirostanes, identified as* epi-*sarsasapogenin and* epi-*smilagenin. Sarsasapogenone was obtained by the oxidation of sarsasapogenin. The reduction of sarsasapogenone using lithium aluminum hydride yielded isomeric products, sarsasapogenin (20%) and* epi-*sarsasapogenin (80%).* (*Steroids* **58**:387–389, 1993)

Keywords: 5β-Spirostane; rumen content of sheep; mass spectrometry; ^{1}H nuclear magnetic resonance spectrometry; ^{13}C nuclear magnetic resonance spectrometry; *epi*-sarsasapogenin; *epi*-smilagenin; steroid

Introduction

Brachiaria decumbens (signal grass), a high yielding grass which is well adapted to the tropical climate,[1,2] is an important source of fodder for ruminant production in Malaysia. However, the grass has been confirmed to be hepatotoxic and nephrotoxic to sheep.[1,3]

It has also been reported that the ethanolic extract of rumen liquor from *B. decumbens* intoxicated sheep contains a hepatotoxic substance or substances causing marked enlargement of the liver and severe necrosis of hepatocytes of rats.[4] The infusion of rumen liquor from *B. decumbens* intoxicated sheep into the rumen of cattle caused hepatic and renal dysfunction, whereas the grass itself when fed directly to cattle did not produce toxic symptoms.[5] These observations suggest strongly that the grass is not toxic per se but contain compounds which as a result of ruminal activities of the sheep were converted to their derivatives responsible for causing the toxicity. This paper describes the identification of two sapogenins isolated from the rumen content of *B. decumbens*-intoxicated sheep.

Address reprint requests to Dr. Nordin H. Lajis, Department of Chemistry, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
Received December 30, 1992; accepted February 1, 1993.

Experimental

General

Melting points were recorded using a Kofler hot bench and were uncorrected. Mass spectra were recorded on Varian MAT CH7 and Finnigan MAT GC-MS SSQ 710 mass spectrometers. Infrared (IR) spectra were recorded with a FTIR 1650 Perkin Elmer spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker CPX 300 spectrometer. Resonances are referenced to TMS.

Reagents

Commercial chromium trioxide, lithium aluminum hydride (BDH reagent grade) and sarsasapogenin (Aldrich) were used without prior purification. Reagent grade pyridine (BDH) was shaken with solid sodium hydroxide and then stored over 5 Å molecular sieves. Commercial methylene chloride was purified by distillation and stored over 5 Å molecular sieves. Silica gel Merck Kieselgel 60 (230 mesh) and Merck Kieselgel 60 PF_{254} were used for column and thin-layer chromatography, respectively.

Oxidation of sarsasapogenin (1)

The oxidation of the title compound using chromium trioxide was carried out according to the published procedure.[6] The crude product sarsasapogenone **(2)** was fractionated on silica gel column to obtain white crystalline product (60% yield) which melted at 222–224 C. Spectral data of sarsasapogenone **(2)** are as follows.

IR (KBr) cm^{-1}. 3411 (br-w), 2953 (br-s), 2859, 1721, 1448, 1380, 1218, 1173, 1134, 1068, 986, 920, 840.

MS, m/z (%). 414 (M^+, 2.5), 335 (1.8), 342 (6.6), 327 (0.6), 300 (6.2), 285 (5.4), 272 (5.6), 271 (24.7), 140 (12.2), 139 (100), 115 (31.2), 107 (6.1), 95 (6.5), 93 (5.6), 81 (6.4), 69 (19.1), 55 (7.8), 43 (3.8).

^{1}H NMR δ (300, MHz, $CDCl_3$). 4.41 (1 H, ddd, J = 8.0, 8.0, 6.4 Hz, C^{16}-H), 3.95 (1 H, dd, J = 11.0, 2.7 Hz, C^{26}-H), 3.30 (1 H, br d, J = 11.0 Hz, C^{26}- H), 2.69 (1 H, dd, J = 14.4, 14.4 Hz, C^4-H), 2.30 (1 H, ddd, J = 14.5, 14.5, 5.3 Hz, C^2-H), 1.08 (3 H, d, J = 7.1 Hz, C^{25}-CH_3), 1.04 (3 H, s, C^{10}-CH_3), 1.00 (3 H, d, J = 6.7 Hz, C^{20}-CH_3), 0.79 (3 H, s, C^{13}-CH_3).

Reduction of sarsasapogenone (2)

The reduction of **2** (0.4 g, 1.0 mmol) was carried out in tetrahydrofuran (THF) using $LiAlH_4$ (22 mmol). The crude yellowish solid product obtained was fractionated on silica gel column to afford 50 mg sarsasapogenin, mp 190–192 C and 300 mg *epi*-sarsasapogenin **(3)** mp 200-201 C. The thin-layer chromatographic analysis on silica gel revealed that the R_f of **1** was 0.34 while that of **3** was 0.22 when $CHCl_3$ was used as developing solvent. The spectral data of *epi*-sarsasapogenin **(3)** are as below.

IR (KBr) cm^{-1}. 3336 (br-s), 2938, 2865, 1654 (br-w), 1448, 1368, 1214 (w), 1172, 1132 (2), 1052, 986, 922, 850.

MS, m/z (%). 416 (M^+, 1.3), 357 (1.7), 344 (4.7), 329 (3.1), 302 (4.9), 287 (5.1), 284 (7.6), 273 (15.1), 255 (11.9), 140 (12.4), 139 (100), 122 (9.2), 115 (18.6), 109 (6.9), 107 (7.5), 95 (7.1), 93 (6.7), 81 (7.3), 69 (17.7), 55 (8.1), 44 (9.9), 43 (6.3).

^{1}H NMR δ (300 MHz, $CDCl_3$). 4.40 (1 H, ddd, J = 8.0, 8.0, 6.4 Hz, C^{16} – H), 3.95 (1 H, dd, J = 11.0, 2.6 Hz, C^{26} – H), 3.64 (1 H, m, β – C^3 – H), 3.30 (1 H, br d, J = 11.0 Hz, C^{26} – H), 1.08 (3 H, d, J = 7.1 Hz, C^{25} – CH_3), 0.99 (3 H, d, J = 6.7 Hz, C^{20} – CH_3), 0.93 (3 H, s, C^{10} – CH_3), 0.75 (3 H, s, C^{13} – CH_3).

Extraction and purification of epi-sarsasapogenin and epi-smilagenin from the rumen contents

The rumen contents of *B. decumbens* intoxicated sheep were collected and dried in the oven at 40–50 C. The dried rumen content was ground and 40 g was extracted with chloroform according to the procedure described by Stahr.[7] Approximately 8 g of crude semi-solid extract was obtained.

Several fractions were separated after column chromatography on silica gel using $CHCl_3$: light petroleum bp 60-80 C (6 : 4) as the eluent. The major fraction was further purified by thin-layer chromatography on silica gel using chloroform-dichloromethane as the solvent. Two compounds, **3** and **4**, each nearly 0.10 g, with mp 199-201 C and 204-206 C, respectively, were isolated. Thin-layer chromatographic analysis on silica gel with $CHCl_3$ as the developing solvent gave R_f for **3** and **4** as 0.22 and 0.25, respectively.

The IR, ^{1}H NMR, and MS spectral data of isolated compounds **3** and **4** were identical with the corresponding spectral data of synthetic *epi*-sarsasapogenin **(3)** and *epi*-smilagenin,[8–10] respectively (Figure 1).

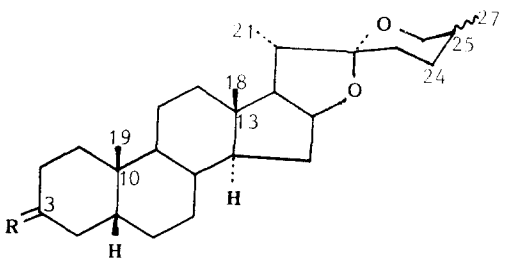

Figure 1 Structures of spirostane and its derivatives:

1, R =  ; (25*S*)-5β-spirostan-3β-ol (sarsasapogenin);

2, R = 0; (25*S*)-5β-spirostan-3-one (sarsasapogenone);

3, R =  ; (25*S*)-5β-spirostan-3α-ol (*epi*-sarsasapogenin);

4, R =  ; (25*R*)-5β-spirostan-3α-ol (*epi*-smilagenin);

5, R =  ; (25*R*)-5β-spirostan-3β-ol (smilagenin);

6, R = ; (25*R*)-5β-spirostane.

Results and discussion

The colorless powder (**3** and **4**) isolated from the rumen contents was shown by mass spectrometry to be isomeric. The mass spectra indicated that these components, **3** and **4**, *M*W 416, which were consistent with the molecular formula $C_{27}H_{44}O_3$, were 3-spirostanols closely related to but not identical with, sarsasapogenin and tigogenin.

The isolation of sarsasapogenone and *epi*-sarsasapogenin from *Dioscorea collettii* have been reported although the structural elucidation has been carried out without ^{13}C NMR and detailed ^{1}H NMR spectra studies.[11] We could not find the exact comparison between the characteristic ^{1}H NMR signals for the isolated[11] and synthesized *epi*-sarsasapogenin. The presence of *epi*-smilagenin β-D-glucuronide in the bile of sheep grazing *Panicum dichotomiflorum* (smooth witch grass)[9,12] and *Panicum schinzii* (sweet grass)[13] has been recently reported.[10] Structure **3** for *epi*-sarsasapogenin was established based on comparison of the spectral data of the synthetic analog derived from sarsasapogenone. The assignment of ^{1}H NMR and ^{13}C NMR data for sarsasapogenone was carried out based on the published data on related compounds.[8,14–16]

The ^{13}C NMR chemical shifts of the compounds **3** and **4** isolated from rumen content are shown in Table 1. The ^{13}C NMR signals of **3** and **4** were consistent with those of our synthesized *epi*-sarsasapogenin and reported *epi*-smilagenin, respectively. The characteristic signal of C^{25} of **4** showed downfield shift by 3.2 ppm as compared to that of **3.** This signal was in turn consistent with that of reported value for *epi*-smilagenin (Table 1).

It is interesting to note that the α-C^3-H of sarsasapo-

Table 1 ^{13}C NMR chemical shifts of spirostanes

Carbon atom	1	2	3	3[a]	4[a]	4[b]	5[c]	6[b]
1	29.9	37.1	35.3	35.3	35.3	35.5	29.9	37.6
2	27.8	36.9	30.4	30.5	30.5	30.5	27.8	21.3
3	67.0	213.1	71.7	71.8	71.8	71.8	67.0	27.0
4	33.5	42.3	36.4	36.4	36.4	36.5	33.6	27.2
5	36.5	44.2	42.0	42.0	42.0	42.1	36.6	43.7
6	26.5	26.0	26.7	26.7	27.1	27.1	26.5	27.4
7	26.5	26.5	27.1	27.1	26.7	26.7	26.5	26.8
8	35.2	35.1	35.4	35.4	35.4	35.5	35.3	35.5
9	39.6	40.7	40.5	40.5	40.5	40.6	40.3	40.6
10	35.2	35.0	34.7	34.7	34.7	34.7	35.3	35.5
11	20.7	20.9	20.6	20.6	20.6	20.6	20.9	20.6
12	40.3	40.1	40.2	40.2	40.2	40.3	39.9	40.3
13	40.7	40.6	40.6	40.6	40.7	40.6	40.7	40.6
14	56.4	56.2	56.3	56.3	56.3	56.4	56.5	56.5
15	31.7	31.6	31.7	31.7	31.8	31.8	31.8	31.7
16	81.0	80.6	81.0	81.0	80.9	80.9	80.9	81.0
17	62.0	62.0	62.0	62.0	62.2	62.3	62.4	62.3
18	16.5	16.4	16.5	16.5	16.4	16.5	16.4	16.4
19	23.9	22.6	23.4	23.4	23.4	23.4	23.8	24.2
20	42.1	42.1	42.1	42.1	41.6	41.6	41.6	41.6
21	14.3	14.3	14.3	14.3	14.5	14.5	14.4	14.5
22	109.7	109.7	109.7	109.7	109.2	109.2	109.1	109.2
23	25.9	25.9	25.9	25.9	31.4	31.4	31.4	31.4
24	25.7	25.7	25.7	25.8	28.8	28.8	28.8	28.8
25	27.1	27.0	27.0	27.1	30.3	30.3	30.3	30.3
26	65.1	65.1	65.1	65.1	66.8	66.8	66.8	66.8
27	16.0	16.0	16.0	16.0	17.1	17.1	17.1	17.1

[a] Obtained from rumen-extract, measured at 75.4 MHz.
[b] From reference 8.
[c] From references 8 and 16.

genin resonated at 4.10 ppm and produced a broad singlet while the β-C^3-H of *epi*-sarsasapogenin, **3** and **4** resonated at considerably higher field (3.64–3.63 ppm) with its coupling to vicinal protons produced a multiplet. The ^1H NMR chemical shift of one of the CH_3 groups attached to a quaternary carbon which is tentatively assigned to the C^{10}-CH_3 protons showed a significant dependence on the substituent and configurational characteristic at C^3 carbon.

The proton spectrum for sarsasapogenone **(2)** has not been reported. However, Drewes et al.[17] have recently reported that the ^1H NMR peaks for 2α-H of 6β-hydroxy-lup-20(30)-en-3-one appeared at δ 2.26 (ddd, J = 15.01, 6.48, 2.85 Hz) and that of 2β-H appeared at δ 2.82 ppm (ddd, J = 15.01, 6.41, 2.91 Hz). Based on this observation the peak at δ 2.30 ppm in the proton spectrum of **2** is then tentatively assigned to 2α-H or 2β-H while that at δ 2.69 ppm is assigned to 4β-H.

A number of 3-spirostanols have been isolated from plants.[18] These include tigogenin, *epi*-tigogenin, sarsasapogenin, neotigogenin, smilagenin, *epi*-smilagenin and *epi*-neotigogenin diastereomer.[19] Sarsasapogenin has been isolated from *Asparagus adscendens* and showed considerable antibacterial activity.[20] It has also been reported that the extract of *Y. shidigera* which also contained sarsasapogenin has been used as an anti-stress agent in poultry and as a growth promotant in cattle.[21]

References

1. Chin FY, Chew ES, Lima E (1979). The growth, yield and compositional characteristic of eleven grasses grown in Malaysia. *Kajian Vet* **11:**1–5.
2. Wong CC (1980). Productivity and chemical composition of 20 improved tropical grasses in the humid tropics. *MARDI Res Bulletin* **8:**163–173.
3. Salam AA, Nordin MM, Rajion MA (1987). Toxicity of *Brachiaria decumbens* in sheep and goats. Paper presented at the 1st Congress of Toxicology in Developing Countries (DC), Buenos Aires, Argentina, November 15–21.
4. Salam AA (1987). Toxic extract of rumen liquor of sheep fed *Brachiaria decumbens*. Proc. IFS Symposium of Science Asia, Kuala Lumpur, October 14–17, p 83–86.
5. Nordin MM, Salam AA, Rajion MA (1989). Experimental *Brachiaria decumbens* toxicity in cattle. *Vet Res Comm* **13:**491–494.
6. Ratcliffe R, Rodehorst R (1970). Improved procedure for oxidation with the chromium trioxide-pyridine complex. *J. Org. Chem.* **35:**4000–4002.
7. Stahr HM (1977). Extraction and qualitative identification of alkaloids. *Analytical Toxicology Methods Manual.* Iowa State University Press, Iowa pp. 178–181.
8. Tori K, Seo S, Terui Y, Nishikawa J, Yasuda F (1981). Carbon-13 NMR spectra of 5β-steroidal sapogenins. Reassignment of the F-ring carbon signals of (25S)-*spirostans*. *Tetrahedron Lett*, 2405–2408.
9. Holland, PT, Miles CO, Mortimer PH, Wilkins AL, Hawkes AD, Smith BL (1991). Isolation of the steroidal sapogenin *epi*-smilagenin from the bile of sheep affected by *Panicum dichotomiflorum* toxicosis. *J Agric Food Chem* **39:**1963–1965.
10. Christopher OM, Alistair LW, Sarah CM, Patrick TH, Barry LS, Malcolm JL, Peter PE (1992). Identification of the calcium salt of *epi*-smilagenin β-D-glucuronide in the bile crystals of sheep affected by *Panicum dichotomiflorum* and *Panicum schinzii* toxicoses. *J Agric Food Chem* **40:**1606–1609.
11. Chenglai L, Yanyong C (1985). Steroidal sapogenins from mildewed *Dioscorea collettii* Hook. F. *Yaoxue Xuebao* **20:**143–145.
12. Miles CO, Munday SC, Holland PT, Smith BL, Embling PP (1991). Identification of a sapogenin glucuronide in the bile of sheep affected by *Panicum dichotomiflorum* toxicosis. *New Zealand Vet J* **39:**150–152.
13. Miles CO, Munday SC, Holland PT, Lancaster MJ, Wilkins AL (1992). Further analysis of bile crystals from sheep grazing *Panicum schinzii* (sweet grass). *Aust Vet J* **69:**34.
14. Atta-ur-Rahman (1990). *Handbook of Natural Products Data*, vol 1, Elsevier, Amsterdam, pp. 383–401, 493–494, 503–506 and references cited therein.
15. Eggert H, Djerassi C (1975). ^{13}C NMR spectra of sapogenins. *Tetrahedron Lett* 3635–3638.
16. Garcia JAR, Castro HTV (1987). ^{13}C NMR spectroscopy of (25R)-5α-spirostanes. *Org Magn Reson* **25:**831–834.
17. Drewes SE, Masimbye MJ, Field JS, Ramesar N (1991). 11,11-Dimethyl-1,3,-8,10-tetrahydroxy-9-methoxypeltogynan and three pentacyclic triterpenes from *cassine transvaalensis*. *Phytochemistry* **30:**3490–3493.
18. Pollock JRA, Steven R, eds. (1965). *Dictionary of Organic Compounds*, vol 5, 4th edn., Eyre & Spottiswoode Publisher Ltd., p. 2913.
19. Blunden G, Dov S, Beach SJ, Turner CH (1984). Australigenin, a new steroidal sapogenin from *Cordyline australis* fruits. J Nat Prod **47:**266–269.
20. Grover GS, Rao JT (1988). Chemical and antimicrobial studies of a saponin of *Asparagus adscendens*. *Fitoterapia* **59:** 415–416.
21. Kaneda N, Nakanishi H, Staba EJ (1987). Steroidal constituents of *Yucca schidigera* plants and tissue cultures. *Phytochemistry* **26:**1425–1429.