Identification of Insoluble Salts of the β-D-Glucuronides of Episarsasapogenin and Epismilagenin in the Bile of Lambs with Alveld and Examination of *Narthecium ossifragum*, *Tribulus terrestris*, and *Panicum miliaceum* for Sapogenins

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Crystalloid material soluble in acetic acid was isolated from the bile of lambs with alveld (a hepatogenous photosensitization disease which develops after grazing *Narthecium ossifragum*). The main components of these bile extracts were shown by hydrolysis, GC–MS, TLC, LSIMS, ^{1}H and ^{13}C NMR, and X-ray analysis to be salts of a 4:1 mixture of episarsasapogenin β-D-glucuronide (1) and epismilagenin β-D-glucuronide (2). The ^{13}C NMR spectrum of 1 was fully assigned by comparison with the NMR spectra of model compounds. A metabolic route for conversion of the sarsasapogenin saponins of *N. ossifragum* into 1 is proposed. Saponins were extracted from the foliage of *N. ossifragum*, *Tribulus terrestris*, and *Panicum miliaceum*. The saponins were hydrolyzed to sapogenins, which were examined by NMR spectroscopy and GC–MS. *P. miliaceum* afforded a 4:1 mixture of diosgenin and yamogenin, *T. terrestris* gave a 5:1 mixture of diosgenin and tigogenin, and *N. ossifragum* yielded a mixture (82:9:5:4) of sarsasapogenin, smilagenin, yamogenin, and another spirostanol, tentatively identified as neotigogenin.

INTRODUCTION

Alveld is a hepatogenous photosensitization disease of lambs grazing pastures containing *Narthecium ossifragum*. The livers of lambs with alveld often contain birefringent crystalloid material in and about the bile ducts and in the heptacytes (Flåøyen et al., 1991a). Similar crystalloid material has been observed in hepatogenous photosensitizations of ruminants associated with the ingestion of at least seven other plant species (Munday et al., 1993), most of which appear to contain steroidal saponins (Munday et al., 1993; Abdullah et al., 1992).

We have recently developed methods for chemical analysis of biliary crystals from animals grazing two species of *Panicum* and were able to show that in both cases the biliary crystals were composed principally of the calcium salt of epismilagenin β-D-glucuronide (Miles et al., 1991, 1992a,b; Holland et al., 1991). We have also established that the major saponins in these two *Panicum* species are derived from diosgenin and that the biliary crystals result from metabolism of these saponins after their ingestion by the sheep (Miles et al., 1992b; Munday et al., 1993). *N. ossifragum* is also known to contain steroidal saponins, although these are derived from sarsasapogenin (Ceh and Hauge, 1981).

We now report the application of these methods to the bile sediment from lambs with alveld, showing it to contain salts of episarsasapogenin β-D-glucuronide (1) and epismilagenin β-D-glucuronide (2) (see Figure 1). We also found that *N. ossifragum* contains saponins derived principally from sarsasapogenin and smilagenin. *Panicum miliaceum* and *Tribulus terrestris*, plant species associated with hepatogenous photosensitizations similar to alveld, were found to contain saponins derived mainly from diosgenin and yamogenin and from diosgenin and tigogenin, respectively.

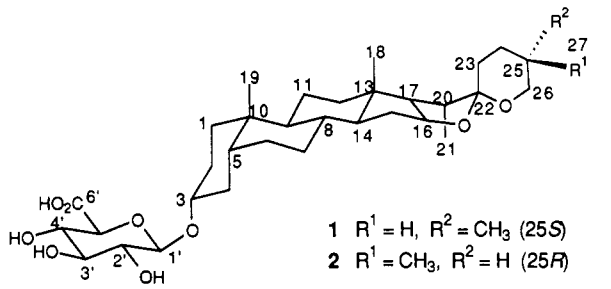

Figure 1. Structures of episarsasapogenin β-D-glucuronide (1) and epismilagenin β-D-glucuronide (2).

MATERIALS AND METHODS

General. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR, liquid surface-assisted ionization mass spectrometry (LSIMS), energy-dispersive X-ray analysis (EDXA), and acidic hydrolysis of biliary crystals were performed as reported elsewhere (Miles et al., 1992b). GC–MS was as described by Miles et al. (1992b) using the following conditions: 25 m × 0.22 mm HP-1 column; temperature program, 170 °C (0.2-min hold) to 240 °C at 35 °C/min and then to 295 °C at 2 °C/min (5-min hold). Under these conditions authentic standards of sarsasapogenin, episarsasapogenin, smilagenin, epismilagenin, tigogenin, and diosgenin eluted at 20.07, 20.07, 19.82, 19.70, 20.45, and 20.20 min, respectively. TLC was performed on silica gel plates (E. Merck 5554) using the following eluents: A, chloroform–methanol, 49:1; B, chloroform–methanol–water, 65:35:10 (bottom layer); C, chloroform–methanol–water, 65:35:10 (bottom layer) plus 1% v/v acetic acid; D, ethyl acetate–petroleum spirit (40–60 °C), 3:7. Plates were visualized by spraying with anisaldehyde reagent (Stahl, 1969) or 5% sulfuric acid in ethanol. HPTLC was performed on silica gel plates (E. Merck 5556) using eluent A.

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Table I. ^{13}C NMR Chemical Shifts (δ) in CD_3CO_2D

	5	2[a]	1
1	36.3	36.0	36.0
2	30.6	27.4	27.4
3	73.1	80.2	80.3
4	36.5	34.9	34.9
5	43.3	43.2	43.2[b]
6	28.1	28.0	28.0
7	27.8	27.6	27.6
8	36.7	36.5	36.5
9	41.7	41.6	41.6
10	35.7	35.7	35.6
11	21.7	21.5	21.5
12	41.2	41.0	41.0
13	41.6	41.5	41.5
14	57.4	57.2	57.2
15	32.4	32.3	32.3
16	82.5	82.2	82.0
17	63.1	63.1	62.9
18	17.0	17.3	16.8
19	23.9	23.8	23.8
20	43.3	42.7	43.1[b]
21	14.8	14.8	14.6
22	111.4	110.8	111.1
23	c	32.0	26.6
24	26.4	29.5	26.4
25	28.2	31.0	28.0
26	66.0	67.5	65.5
27	16.5	16.8	16.3
1′		101.4	101.5
2′		74.2	74.2
3′		76.6	76.6
4′		72.4	72.4
5′		75.1	75.2
6′		173.2	173.4

[a] From Miles et al. (1992b). [b] Assignments are interchangeable. [c] Not observed (deuterium exchanged, see text).

Collection of Bile. Bile was collected from 10 lambs which showed photosensitization while grazing pastures on the west coast of Norway which contained *N. ossifragum*. Lambs were killed by an overdose of pentobarbitone. Bile was collected from the gall bladder and centrifuged, and the supernatant was discarded. The precipitate was air-dried, giving a dark green solid (ca. 100 mg).

Isolation of Sapogenin Glucuronides. The bile precipitate was extracted as described previously for biliary crystals from sheep grazing *Panicum schinzii* (Miles et al., 1992a,b) to afford a pale green solid. This extract was purified by flash chromatography (Still et al., 1978) using eluent B (100 mL) followed by eluent C, to afford sapogenin derivatives as a cream-colored solid (2.6 mg). The major component was found to be episarsasapogenin β-D-glucuronide (1) by LSIMS and NMR analysis. LSIMS of 1 showed a quasimolecular ion at m/z 593 in positive ion mode with a prominent fragment at m/z 417 and a quasimolecular ion at m/z 591 in negative ion mode. ^1H NMR (CD_3CO_2D) δ 0.78 (s, CH_3), 0.95 (s, CH_3), 1.00 (d, J = 6.6 Hz, $CHCH_3$), 1.06 (d, J = 6.9 Hz, $CHCH_3$), 3.32 (br d, J = 10.2 Hz, H-26α), 3.45 (~t, J = 8.2 Hz, H-2′), 3.70 (~t, J = 8.9 Hz, H-3′), 3.74 (m, H-3β), 3.78 (~t, J = 9.3 Hz, H-4′), 3.93 (br d, J = 10.2 Hz, H-26β), 3.99 (d, J = 9.5 Hz, H-5′), 4.45 (br q, J = 6.5 Hz, H-16), 4.61 (d, J = 7.8 Hz, H-1′); ^{13}C NMR (CD_3CO_2D) δ, see Table I. The minor component was identified by ^1H and ^{13}C NMR spectroscopy as epismilagenin β-D-glucuronide (2) by comparison with spectra of an authentic sample (Miles et al., 1992b).

Episarsasapogenin (5). Sarsasapogenin (101.6 mg) in dichloromethane was oxidized by addition of an excess of pyridinium chlorochromate. The reaction was complete (TLC, eluent A) after 24 h. The reaction mixture was filtered to remove precipitates, diluted to 50 mL with dichloromethane, washed with water, and dried ($MgSO_4$), and the solvent was removed in vacuo. Purification by flash chromatography using chloroform as the eluent gave sarsasapogenone (4) (67.1 mg) as a colorless solid; mp 212–215 °C; ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3), 0.99 (d, J = 6.5 Hz, $CHCH_3$), 1.03 (s, CH_3), 1.07 (d, J = 6.8 Hz, $CHCH_3$), 3.30 (br d, J = 10.3 Hz, H-26α), 3.94 (br d, J = 10.3 Hz, H-26β), 4.21 (br q, J = 7 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 14.4 (q), 16.1 (q), 16.5 (q), 21.1 (t), 22.7 (q), 25.8 (t), 26.0 (t), 26.1 (t), 26.6 (t), 27.1 (d), 31.7 (t), 35.1 (s), 35.2 (d), 37.0 (t), 37.2 (t), 40.2 (t), 40.7 (s), 40.9 (d), 42.2 (d), 42.4 (t), 44.3 (d), 56.3 (d), 62.1 (d), 65.2 (t), 80.9 (d), 109.8 (s), 213.2 (s).

Reduction of 4 (25.9 mg) using $NaBH_4$ (Blunden et al., 1979) followed by purification using flash chromatography (eluent D) gave episarsasapogenin (5) (22.5 mg) as a colorless solid, mp (ethanol–water) 209–211 °C; ^1H NMR (C_5D_5N) δ 0.84 (s, CH_3), 0.92 (s, CH_3), 1.09 (d, J = 7.0 Hz, $CHCH_3$), 1.16 (d, J = 6.9 Hz, $CHCH_3$) 3.37 (d, J = 10.9 Hz, H-26α), 3.86 (m, H-3β), 4.07 (dd, J = 2.6, 10.9 Hz, H-26β), 4.58 (br q, J = 6.6 Hz, H-16); ^{13}C NMR (C_5D_5N) δ 14.9 (q), 16.3 (q), 16.7 (q), 20.9 (t), 23.7 (q), 26.2 (t), 26.4 (t), 27.0 (t), 27.6 (t), 27.6 (d), 31.4 (t), 32.2 (t), 35.0 (s), 35.7 (d), 36.0 (t), 37.3 (t), 40.3 (t), 40.9 (s), 40.9 (d), 42.5 (d), 42.5 (d), 56.5 (d), 63.0 (d), 65.1 (t), 70.1 (d), 81.3 (d), 109.6 (s); ^{13}C NMR (CD_3CO_2D) δ, see Table I.

Isolation of Plant Sapogenins. *N. ossifragum* was obtained from pastures on the west coast of Norway (Flåøyen et al., 1991b). *P. miliaceum* was grown from seed at Ruakura, and foliage was harvested shortly before seed maturation. The plant material was freeze-dried on the day of collection and then ground. *T. terrestris* was harvested in March 1985 in Victoria, Australia, and air-dried before grinding.

Plant saponins were extracted and hydrolyzed, and the crude sapogenin fraction was isolated using the method of Miles et al. (1992b). The sapogenin fraction was taken up in dichloromethane (1 mL) and applied to a silica gel Sep-Pak cartridge (Waters) that had been prewashed with dichloromethane. After elution with dichloromethane (3 mL) to remove 3,5-spirostadienes, the sapogenin fraction was eluted with dichloromethane–methanol (49:1, 3 mL) and concentrated in vacuo. NMR spectroscopy and GC–MS showed that only spirostan-3-ols were present.

N. ossifragum extract GC–MS showed peaks at 19.65 (smilagenin), 20.15 (sarsasapogenin), and 20.65 min [a 5:4 mixture of a spirostanol tentatively identified as neotigogenin (M^+ 416 daltons) and yamogenin (M^+ 414 daltons), respectively] (TIC integral ratio 9:82:9). ^1H and ^{13}C NMR indicated the presence of sarsasapogenin and smilagenin in a ratio of ca. 9:1. Sarsasapogenin (the major component): ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3), 0.97 (s, CH_3), 0.98 (d, J = 6.5 Hz, $CHCH_3$), 1.07 (d, J = 6.8 Hz, $CHCH_3$), 3.29 (d, J = 12.2 Hz, H-26α), 3.94 (dd, J = 2.6, 12.2 Hz, H-26β), 4.10 (m, H-3), 4.40 (br q, J = 6.6 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 14.4 (q), 16.1 (q), 16.5 (q), 20.9 (t), 24.0 (q), 25.8 (t), 26.0 (t), 26.6 (t), 26.6 (t), 27.1 (d), 27.9 (t), 30.0 (t), 31.8 (t), 33.6 (t), 35.3 (d), 35.3 (s), 36.6 (d), 39.9 (t), 40.4 (t), 40.7 (s), 42.2 (t), 56.5 (d), 62.2 (d), 65.2 (t), 67.2 (d), 81.1 (d), 109.8 (s). Smilagenin (the minor component): ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3), 0.78 (d, J = 6.3 Hz, $CHCH_3$), 0.96 (d, J = 6.7 Hz, $CHCH_3$), 0.97 (s, CH_3), 3.37 (t, J = 10.7 Hz, H-26α), 3.47 (m, H-26β), 4.10 (m, H-3), 4.40 (br q, J = 6.6 Hz, H-16). Some of the ^{13}C NMR signals attributable to smilagenin [see Agrawal et al. (1985)], including ^{13}C NMR ($CDCl_3$) δ 14.6 (C-21), 16.5 (C-18), 17.2 (C-27), 28.9 (C-24), 30.3 (C-25), 31.4 (C-23), 41.7 (C-20), 62.3 (C-17), and 66.9 (C-26), were adequately distinguished from those of sarsasapogenin.

P. miliaceum extract GC–MS showed peaks at 20.27 (diosgenin) and 20.57 min (yamogenin) (TIC integral ratio 79:21). ^{13}C NMR revealed diosgenin and yamogenin to be present in a ratio of ca. 4:1. Diosgenin: ^{13}C NMR ($CDCl_3$) δ 14.6 (q), 16.3 (q), 17.2 (q), 19.5 (q), 20.9 (t), 28.6 (t) 30.4 (d), 31.4 (t), 31.5 (t), 31.8 (t), 31.9 (t), 32.1 (t), 36.7 (s), 37.3 (t), 39.8 (t), 40.3 (s), 41.7 (d), 42.3 (t), 50.1 (d), 56.6 (d), 62.2 (d), 66.9 (t), 71.8 (d), 80.9 (d), 109.3 (s), 121.5 (d), 140.8 (s). Yamogenin: ^{13}C NMR ($CDCl_3$) δ 14.4 (q), 16.1 (q), 16.3 (q), 19.5 (q), 20.9 (t), 25.8 (t), 26.0 (t), 27.1 (d), 31.5 (d), 31.8 (t), 31.9 (t), 32.1 (t), 36.7 (s), 37.3 (t), 39.8 (t), 40.3 (s), 42.2 (d), 42.3 (t), 50.1 (d), 56.6 (d), 62.0 (d), 65.2 (t), 71.8 (d), 81.0 (d), 109.9 (s), 121.5 (d), 140.8 (s).

T. terrestris extract GC–MS showed peaks at 20.30 (diosgenin) and 20.45 min (tigogenin) (TIC integral ratio 84:16). ^1H and ^{13}C NMR revealed the presence of diosgenin and tigonenin in a ratio of ca. 5:1. Diosgenin: ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3), 0.79 (d, J = 6.0 Hz, $CHCH_3$), 0.97 (d, J = 6.8 Hz, $CHCH_3$), 1.02 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 14.6 (q), 16.3 (q), 17.2 (q), 19.5 (q), 20.9 (t), 28.6 (t), 30.4 (d), 31.4 (t), 31.5 (t), 31.8 (t), 31.9 (t), 32.1 (t), 36.7 (s), 37.3 (t), 39.8 (t), 40.3 (s), 41.7 (d), 42.3 (t), 50.1 (d), 56.6 (d), 62.2 (d), 66.9 (t), 71.8 (d), 80.9 (d), 109.3 (s), 121.5 (d), 140.8 (s). Tigogenin: ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3), 0.79 (d, J = 6.2 Hz, $CHCH_3$), 0.81 (s, CH_3), 0.95 (d, J = 6.8 Hz, $CHCH_3$). Some of

the ^{13}C NMR signals attributable to tigogenin [see Agrawal et al. (1985)], including ^{13}C NMR ($CDCl_3$) δ 71.3 (C-3), 54.4 (C-9), 44.9 (C-5), 35.2 (C-8), 21.1 (C-11), and 16.5 (C-18), were adequately differentiated from those of diosgenin.

Chemicals. Diosgenin was obtained from Sigma Chemical Co. and epismilagenin from Steraloids Inc. Tigogenin, smilagenin, and sarsasapogenin (3) were obtained from Upjohn Laboratories. The authenticity of these sapogenin samples was established by NMR spectroscopy (Agrawal et al., 1985). The calcium salt of epismilagenin β-D-glucuronide was available from previous work (Miles et al., 1992b).

RESULTS AND DISCUSSION

Biliary Crystals. Examination of the isolated bile saponin by LSIMS in positive and negative ion modes indicated a molecular weight of 592. The fragment ion at m/z 417 in the positive ion LSIMS corresponds to loss of glucuronic acid from the quasimolecular ion. The LSIMS is thus identical to that reported for epismilagenin β-D-glucuronide (2) isolated from biliary crystals of sheep grazing both *P. dichotomiflorum* and *P. schinzii* (Miles et al., 1992a,b).

TLC analysis of the bile saponins revealed only one spot, with the same R_f as an authentic sample of the calcium salt of 2, in both eluents B and C. Acidic hydrolysis of the biliary extract gave a sapogenin fraction. Analysis by TLC (eluent D) and HPTLC revealed one spot, which cochromatographed with 5 and epismilagenin, but not with 3, diosgenin, smilagenin, or tigogenin standards. GC–MS of the hydrolysate was consistent with the presence of 5 and epismilagenin in a ratio of 4:1.

^1H and ^{13}C NMR spectroscopy in CD_3CO_2D revealed the presence of two sapogenin glucuronides, in a 4:1 ratio. The ^{13}C NMR spectrum of the minor isomer was identical to that of 2 isolated from *Panicum*-derived biliary crystals (Miles et al., 1992b). The major compound was identified as 1, the 25*S* stereoisomer of 2, by comparison with the literature (Agrawal et al., 1985; Miles et al., 1992b) and with the ^{13}C NMR chemical shifts of 5 (Table I). Only 26 of the expected 27 ^{13}C resonances of 5 were observed in this solvent. The missing resonance can be ascribed to deuterium exchange of the H-23 protons in a manner analogous to that previously observed for epismilagenin in CD_3CO_2D (Miles et al., 1992b).

EDXA of the alveld bile extract indicated the presence of Ca, S, and Na. The solubility characteristics of the extracted bile saponins were identical to those of the biliary crystals from sheep with *P. dichotomiflorum* and *P. schinzii* toxicoses. The biliary crystals associated with these *Panicum* toxicoses have been shown to be calcium salts of 2 (Miles et al., 1992b). We therefore believe that the extracted alveld bile saponins are calcium salts of 1 and 2.

N. ossifragum. We found sarsasapogenin, identified by comparison of its retention time and fragmentation pattern upon GC–MS with that of authentic 3 and by comparison of its ^{13}C NMR resonances with those in the literature (Agrawal et al., 1985), to be the major sapogenin in *N. ossifragum*. The ^1H NMR spectral features of the major constituent of the *N. ossifragum* extract also corresponded exactly with those determined for an authentic specimen of sarsasapogenin.

A minor component (ca. 10% of 3) which exhibited the same GC–MS retention time and fragmentation pattern as smilagenin was also detected in the GC–MS chromatogram. The ^{13}C and ^1H NMR spectra also included a set of signals at ca. 10% of the intensity of the major compound (3), diagnostic for smilagenin. Additionally, the intensity ratios of the pairs of ions at m/z 284/302 and 255/273 in the mass spectrum (Blunden et al., 1980; Holland et al., 1991) were consistent with the identification of this component as smilagenin.

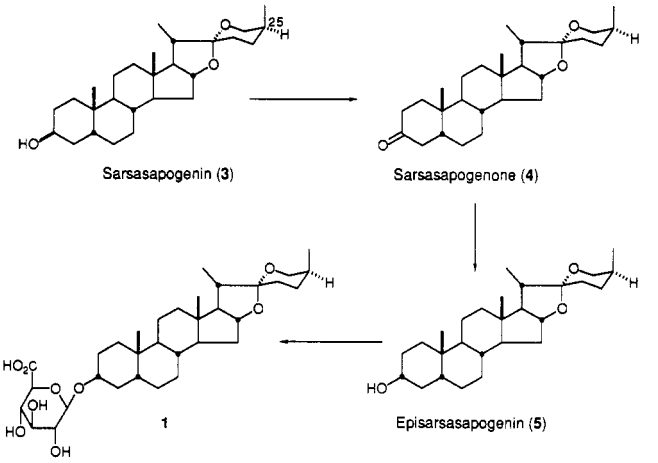

Figure 2. Possible metabolic transformations involved in the conversion of 3 into episarsasapogenin β-D-glucuronide (1).

One other peak, of about the same integrated peak area as the smilagenin peak, was also present in the GC–MS chromatogram. Selected ion monitoring at m/z 414 and 416 revealed it to be due to a closely eluting mixture of a spirostanol (M^+ 416 daltons) and a spirostenol (M^+ 414 daltons). By appropriate subtraction procedures, the mass spectra of the two compounds were obtained. The spirostenol had the same retention time and mass spectral fragmentation pattern as yamogenin (the minor spirostenol from *P. miliaceum*, see below) and is therefore identified as yamogenin. The spirostanol peak eluted slightly later than tigogenin, and its mass spectrum exhibited ratios for the pairs of ions at m/z 284/302 and 255/273 characteristic (Blunden et al., 1980; Holland et al., 1991) of a 3β-hydroxy-5α- or a 3α-hydroxy-5β-spirostanol. These observations suggest the minor spirostanol to be neotigogenin (the 25*S* isomer of tigogenin). This identification, while tentative, is consistent with the preponderance of 25*S* spirostanols in this plant.

The saponins of *N. ossifragum* are therefore derived mainly from 3 but also from smilagenin, yamogenin, and (probably) neotigogenin. Saponins derived from these minor sapogenins have not been detected in previous studies of the saponins from *N. ossifragum* (Ceh and Hauge, 1981), probably because the crystallization steps used during the purification procedures resulted in the loss of the minor components.

This finding is consistent with the presence of 1 and 2, derived by metabolism of the plant saponins, in the alveld biliary sediment. This led us to propose a scheme for the conversion of ingested sarsasapogenin saponins from *N. ossifragum* into 1 by ruminal and tissue metabolism (Figure 2). This scheme closely parallels those previously proposed (Miles et al., 1991, 1992b) for the conversion of diosgenin saponins from *P. dichotomiflorum* and *P. schinzii* into 2. The presence of 2 in the bile of lambs with alveld is presumably due to a metabolic pathway analogous to that shown in Figure 2 but involving the smilagenin saponins now known to be present in *N. ossifragum*.

P. miliaceum. *P. miliaceum* is a grass that is reported (Clare, 1955) to cause hepatogenous photosensitization of sheep accompanied by biliary crystals of the type seen in other *Panicum* toxicoses (Holland et al., 1991). The GC–MS chromatogram of the sapogenin fraction from *P. miliaceum* contained a major and a later-eluting minor peak, both with identical mass spectra. The major compound had the same retention time and fragmentation pattern as diosgenin. Examination of the ^1H and ^{13}C NMR

spectra revealed that a major and a minor compound were present, with chemical shifts identical to those recorded in the literature (Agrawal et al., 1985) for diosgenin and yamogenin, respectively. Two-dimensional ^{1}H NMR analysis of the sapogenin extract verified the existence of mutual couplings between the H-26α and H-26β protons of the respective 25*R* (3.37 and 3.45 ppm, diosgenin) and 25*S* (3.29 and 3.94 ppm, yamogenin) isomers, together with long-range ^4J coupling to their respective H-27 (C-25 methyl group) signals (0.78 ppm, diosgenin; 1.07 ppm, yamogenin). These observations demonstrate that the saponins in *P. miliaceum* are derived from diosgenin and also from yamogenin.

T. terrestris. In a similar manner, ^{1}H and ^{13}C NMR and GC–MS analysis of the *T. terrestris* extract established the presence of diosgenin and tigogenin in a ratio of 5:1. The ^{1}H NMR spectrum of the mixture included tertiary (singlet) methyl group signals at 0.79 and 1.02 ppm (diosgenin, major component) and 0.75 and 0.81 ppm (tigogenin, minor component), the chemical shifts of which corresponded exactly with those determined for authentic specimens of diosgenin and tigogenin, respectively. Although many saponins, including some derived from diosgenin, tigogenin, and neotigogenin, have been isolated from this plant (Mahato et al., 1982), the relative abundances of the spirostanol moieties have not previously been determined.

Conclusions. Biliary crystals from three plant-associated (*N. ossifragum*, *P. dichotomiflorum*, and *P. schinzii*) hepatogenous photosensitizations of sheep have now been identified as insoluble salts of sapogenin glucuronides. In all three cases the structures of the sapogenin glucuronides are consistent with metabolism of the plant saponin(s) before their precipitation in the bile as calcium salts. The key steps are epimerization at the 3-position followed by glucuronide conjugation. *P. miliaceum* is now known to contain saponins similar to those which have been found in other plant species associated with hepatogenous photosensitization. These results indicate that dietary steroidal saponins are essential for the development of the biliary crystals that characterize this group of diseases. Whether or not steroidal saponins are the sole cause of the liver damage has yet to be ascertained; although in view of the reported difficulties experienced in reproducing these diseases by dosing with crude saponins alone (Flåøyen et al., 1991b; Kellerman et al., 1991), it is possible that other factors are involved.

If the route proposed here and elsewhere (Miles et al., 1991, 1992b) for the conversion of plant saponins into biliary crystals is a general one, then knowledge of the spirostanol sapogenins present in a plant would permit a prediction of the chemical nature of the associated biliary crystals to be made. On this basis, we anticipate that biliary crystals from sheep with geeldikkop (*T. terrestris* intoxication) will be composed mainly of epismilagenin β-D-glucuronide along with small amounts of epitigogenin β-D-glucuronide, present as their calcium salts. Similarly, biliary crystals from sheep with *P. miliaceum* intoxication would be expected to consist mainly of the calcium salt of epismilagenin β-D-glucuronide, along with lesser amounts of its 25*S* (i.e., episarsasapogenin) isomer.

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