# Triterpenoid Saponins from Gouania lupuloides

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# TRITERPENOID SAPONINS FROM *GOUANIA LUPULOIDES*[1]

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ABSTRACT.—The stems of Jamaican chawstick, *Gouania lupuloides*, have yielded two novel 16,17-*seco*-dammaranoid saponins, designated gouanoside A [**4**] and gouanoside B [**5**]. Structural assignments are based on spectroscopic data including 2D nmr experiments on the corresponding aglycones, gouanogenin A [**1**] and gouanogenin B [**3**].

*Gouania lupuloides* (L.) Urban (Rhamnaceae) is a neotropical vine that has been used as an implement for teeth cleaning among African-Jamaicans for at least 200 years (1). In traditional use, a pencil-size piece of the bitter tasting vine is chewed on one end, causing extensive foaming and forming a "brush" to rub the teeth and massage the gums (2). Plant extracts are in commerce as a mouthwash and dentrifice throughout Jamaica (3). Although an initial chemical classification of this genus has been conducted (4–6) and the foaming properties of extracts briefly investigated (personal communication, Dr. S. Chilton), no specific compounds have been reported. This paper describes the isolation and structure elucidation of two novel triterpenoid saponins.

## RESULTS AND DISCUSSION

Ground stems of *G. lupuloides* were extracted with MeOH, and the crude saponin complex was obtained by standard solvent partitioning (7). Subsequent chromatography over Si gel produced a band that gave positive color tests for saponins (8,9) and caused hemolysis of red blood cells (9). Although this band appeared homogeneous in a number of tlc systems, spectroscopic data showed that it was still a mixture. In order to characterize the aglycone(s) involved, this mixture was subjected to acid-catalyzed hydrolysis, furnishing the sapogenin gouanogenin A [**1**]. The eims of **1** exhibited a molecular ion at *m/z* 470, shown by a high resolution measurement to be $C_{30}H_{46}O_4$, and a base peak at *m/z* 109. Ir absorptions identified the four oxygens as hydroxyl (3500 $cm^{-1}$), γ-lactone (1772 $cm^{-1}$), and conjugated ketone (1685, 1654, and 1628 $cm^{-1}$). The $^{13}C$ nmr for **1** confirmed these functional groups with resonances at δ 203.2, 177.0, and 78.5; the full spectrum of **1** was strikingly similar to that of the known triterpenoid secondary sapogenin, ebelin lactone [**2**] (10). As the data in Table 1 show, the spectrum of **1** lacked the resonances characteristic of the triene system of **2** (δ 137.0, 135.2, 134.9, 131.6, 126.3, and 124.7) and exhibited instead resonance signals for a new saturated carbon at δ 44.0 and at δ 203.2, 149.0, 140.2, 125.0, and 124.2 indicative of an α,β,γ,δ-unsaturated ketone, in agreement with a uv λ max at 295 nm. $^1H$-nmr resonances (at 600 MHz), δ 7.49 (dd, $J = 11.7$ and 15 Hz), 6.04 (d, $J = 15$

[1]A preliminary report of this work was presented at the International Research Congress on Natural Products, Chicago IL, July 1991: E.J. Kennelly, R.E.K. Winter, M. Elvin-Lewis, J. Gossling, and W.H. Lewis, "Abstracts, 32nd Annual Meeting of the American Society of Pharmacognosy," P-23 (1991).

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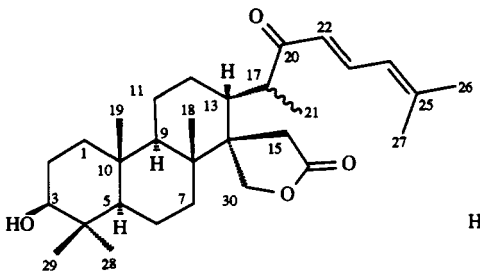
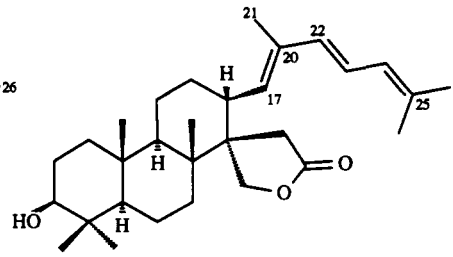


1



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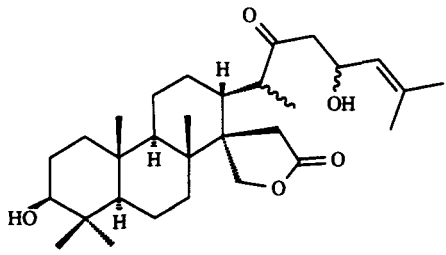


3

Hz), and 5.96 (d, $J$ = 11.7 Hz), defined the dienone system as δ,δ-disubstituted, likely dimethyl ($Me_2C$=CH-CH=CH-CO-), to account for the base peak in the eims.

Recognizing the ebelin lactone is considered an artifact, formed only on acid-catalyzed hydrolysis of various dammarane saponins (10–18), we attempted to effect enzymatic hydrolysis of the saponin mixture using naringinase (15–17). The major hydrolysis product was a new aglycone, gouanogenin B [**3**], although **1** was also detected by tlc. The $^{13}$C-nmr spectrum of **3** corresponds closely to that of **1** excepting the downfield resonances characteristic of the dienone which were replaced by signals for a saturated ketone (δ 213.2) and an isolated trisubstituted double bond (δ 135.7 and 125.9), and upfield signals at δ 65.4 and 48.0; the 600-MHz $^1$H-nmr spectrum of **3** exhibited signals at δ 5.16 (d, $J$ = 8.8 Hz), 4.76 (td, $J$ = 8.8, 3.2 Hz), 2.67 (dd, $J$ = 16.7, 8.8 Hz), and 2.51 (dd, $J$ = 16.7, 3.2 Hz). These data are consistent with the unit $Me_2C$=CH-CH(OH)$CH_2$-CO-, and as expected for a β-hydroxy ketone, conversion of **3** to **1** occurred readily with either acid or base.

Thus it appeared that **1** and **3** differed from ebelin lactone only in the nature of the side chain; the triene unit of **2**, with a methyl group, C-21, attached at C-20, replaced in **1** and **3** by a C-20 carbonyl and the methyl group shifted to C-17. The $J$-coupling networks revealed by total correlation spectroscopy (TOCSY) affirmed this novel carbon skeleton; the data are shown in Table 2. All relays established through vicinal proton-proton coupling were evident; in particular those grouping H-24, H-23, and H-22, as well as Me-21, H-17, H-13, H-12, and H-11, clearly support the connectivities required for structures **1** and **3**.

Chemically pure saponins could be obtained only through preparative hplc, repeated injections finally giving the chromatographically homogeneous saponins, gouanoside A [**4**] and gouanoside B [**5**], the latter the major compound in the purified saponin complex. Their $^{13}$C-nmr spectra (Table 1) were similar to those of **1** and **3**, respectively, except for a downfield displacement of the C-3 resonance by greater than 11 ppm, thus identifying the position of attachment of the sugar chain (18). Additionally, both saponins displayed 12 new carbon resonances, including downfield signals charac-

TABLE 1. $^{13}$C-nmr Assignments for Compounds **1–6**.

| Carbon | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **1**[a] | **2**[b] | **3**[a] | **4**[c] | **5**[c] | **6**[c] |
| C-1 | 38.3 | 38.7 | 38.5 | 38.9 | 39.0 | 38.9 |
| C-2 | 27.1 | 28.0 | 27.3 | 26.9[d] | 26.9[d] | 26.7 |
| C-3 | 78.5 | 77.8 | 78.6 | 90.1 | 90.1 | 90.1 |
| C-4 | 38.7 | 39.4 | 38.9 | 39.8 | 39.8 | 39.7 |
| C-5 | 54.9 | 55.2 | 55.0 | 55.6 | 55.6 | 55.4 |
| C-6 | 17.9 | 18.1 | 18.1 | 18.7 | 18.6 | 18.5 |
| C-7 | 34.4 | 34.5 | 34.7 | 35.0 | 35.1 | 35.0 |
| C-8 | 40.9 | 40.2 | 41.1 | 41.9 | 41.9 | 41.8 |
| C-9 | 52.8 | 52.7 | 53.0 | 53.2 | 53.3 | 53.2 |
| C-10 | 36.9 | 37.3 | 37.1 | 37.5 | 37.5 | 37.4 |
| C-11 | 20.3 | 20.1 | 20.4 | 21.2 | 21.1 | 21.0 |
| C-12 | 24.9 | 28.6 | 24.6 | 25.3[d] | 24.9[d] | 24.8 |
| C-13 | 38.1 | 39.5 | 37.4 | 39.3 | 37.8 | 37.7 |
| C-14 | 52.3 | 52.0 | 51.9 | 53.1 | 52.9 | 52.9 |
| C-15 | 34.2 | 35.0 | 34.3 | 34.9 | 34.9 | 34.9 |
| C-16 | 177.0 | 176.7 | 176.9 | 178.6 | 178.8 | 179.2 |
| C-17 | 44.0 | 131.6[d] | 46.4 | 44.7 | 47.1 | 47.0 |
| C-18 | 18.3 | 18.3 | 18.3 | 18.6 | 18.3 | 18.2 |
| C-19 | 15.3[d] | 16.1 | 15.4[d] | 16.3[e] | 16.4[e] | 16.2 |
| C-20 | 203.2 | 135.2[e] | 213.2 | 204.9 | 213.4 | 213.2 |
| C-21 | 13.8 | 13.3 | 12.4 | 13.9 | 12.0 | 11.8 |
| C-22 | 125.0 | 134.9[d] | 48.0 | 124.8 | 49.1 | 49.0 |
| C-23 | 140.2 | 124.7[d] | 65.4 | 140.5 | 65.8 | 65.6 |
| C-24 | 124.2 | 126.3[d] | 125.9 | 126.6 | 127.9 | 127.4 |
| C-25 | 149.0 | 137.0[e] | 135.7 | 149.9 | 135.2 | 135.4 |
| C-26 | 26.7 | 26.1 | 25.8 | 26.8 | 25.9 | 25.8 |
| C-27 | 19.1 | 18.3 | 18.6 | 19.3 | 18.7 | 18.5 |
| C-28 | 27.9 | 28.6 | 28.1 | 28.1 | 28.2 | 28.0 |
| C-29 | 15.8[d] | 16.2 | 16.0[d] | 16.7[e] | 16.8[e] | 16.6 |
| C-30 | 70.3 | 69.6 | 70.1 | 71.5 | 71.3 | 71.3 |
| Glucose | | | | | | |
| C-1′ | | | | 106.1 | 106.1 | 105.7 |
| C-2′ | | | | 74.9 | 74.8 | 74.7 |
| C-3′ | | | | 77.4 | 77.3 | 77.0 |
| C-4′ | | | | 70.9 | 70.8 | 70.6 |
| C-5′ | | | | 75.7 | 75.5 | 76.6 |
| C-6′ | | | | 67.7 | 67.5 | 62.0 |
| Rhamnose | | | | | | |
| C-1″ | | | | 101.4 | 101.3 | |
| C-2″ | | | | 71.4 | 71.4 | |
| C-3″ | | | | 71.9 | 71.9 | |
| C-4″ | | | | 73.4 | 73.4 | |
| C-5″ | | | | 69.1 | 69.1 | |
| C-6″ | | | | 18.2 | 18.2 | |

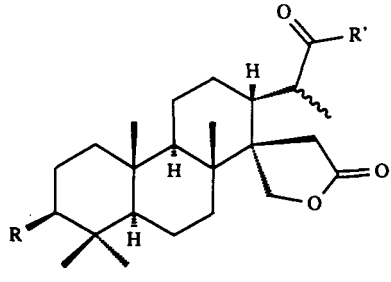
[a]In $CDCl_3$.
[b]In $C_5D_5N$. Data are from Kobayashi *et al.* (10).
[c]In $CD_3CN/D_2O$.
[d,e]Assignments interchangeable in same column.

teristic of anomeric carbons near 106 and 101 ppm and that of a methyl group at δ 18.2, showing two hexoses, one a 6-deoxy sugar. Comparisons with data for model glycosides (19) indicated glucose and rhamnose as most likely, and these were the only

TABLE 2. $^1$H nmr Data for Compounds **1** and **3** (600 MHz, $CDCl_3$).

| Proton (COSY, HMQC) | Compound | | | |
|---|---|---|---|---|
| | **1** | | **3** | |
| | δ ppm[a] | $^1$H/$^1$H Relays (TOCSY) | δ ppm[a] | $^1$H/$^1$H Relays (TOCSY) |
| H-1 | 0.85 m | 1.68, 1.50, 1.58, 3.14 | 0.88 m | 1.71, 1.48, 1.56, 3.17 |
| | 1.68 m | 0.85, 1.50, 1.58, 3.14 | 1.71 m | 0.88, 1.48, 1.56, 3.17 |
| H-2 | 1.50 m | 0.85, 1.68, 1.58, 3.14 | 1.48 m | 1.56, 3.17, 0.88, 1.71 |
| | 1.58 m | 0.85, 1.68, 1.50, 3.14 | 1.56 m | 1.48, 3.17, 0.88, 1.71 |
| H-3 | 3.14 dd (11.5, 4.5) | 0.85, 1.68, 1.50, 1.58 | 3.17 dd (11.6, 4.5) | 0.88, 1.71, 1.48, 1.56 |
| H-5 | 0.62 d (11.4) | 1.39, 1.58, 1.45, 1.30 | 0.65 dd (12.0, 1.8) | 1.43, 1.62, 1.50, 1.35 |
| H-6 | 1.39 m | 0.62, 1.58, 1.45, 1.30 | 1.43 m | 1.62, 0.65, 1.50, 1.35 |
| | 1.58 m | 0.62, 1.39, 1.45, 1.30 | 1.62 m | 1.43, 0.65, 1.50, 1.35 |
| H-7 | 1.45 m | 1.30, 1.39, 1.58, 0.62 | 1.50 m | 1.35, 1.43, 1.62, 0.65 |
| | 1.30 m | 1.45, 1.39, 1.58, 0.62 | 1.35 m | 1.50, 1.43, 1.62, 0.65 |
| H-9 | 0.60 dd (12.0, 2.5) | 1.32, 1.51, 1.08, 1.68, 2.38, 2.61, 1.02 | 0.61 dd (12.6, 3.0) | 1.36, 1.57, 1.13, 1.61, 2.41, 2.50, 1.03 |
| H-11 | 1.32 m | 1.51, 0.60, 1.08, 1.68, 2.38, 2.61, 1.02 | 1.57 m | 1.36, 0.61, 1.13, 1.61, 2.41, 2.50, 1.03 |
| | 1.51 m | 1.32, 0.60, 1.08, 1.68, 2.38, 2.61, 1.02 | 1.36 m | 1.57, 0.61, 1.13, 1.61, 2.41, 2.50, 1.03 |
| H-12 | 1.08 m | 1.68, 2.38, 2.61, 1.02, 1.32, 1.51, 0.60 | 1.13 qd (13.2, 4.8) | 1.61, 1.36, 1.57, 0.61, 2.41, 2.50, 1.03 |
| | 1.68 m | 1.08, 2.38, 2.61, 1.02, 1.32, 1.51, 0.60 | 1.61 m | 1.13, 1.36, 1.57, 0.61, 2.41, 2.50, 1.03 |
| H-13 | 2.38 dbt (13.2, 4.2) | 2.61, 1.02, 1.08, 1.68, 1.32, 1.51, 0.60 | 2.41 dbt (13.5, 3.5) | 2.50, 1.03, 1.61, 1.13, 1.57, 1.36, 0.61 |
| H-15 | 2.03 d (19) | 2.51 | 2.59 d (19) | 2.12 |
| | 2.51 d (19) | 2.03 | 2.12 d (19) | 2.59 |
| H-17 | 2.61 qd (7.2, 4.2) | 1.02, 2.38, 1.68, 1.08, 1.32, 1.51, 0.60 | 2.50 qd (7.0, 3.5) | 1.03, 2.41, 1.61, 1.13, 1.57, 1.36, 0.61 |
| H-18 | 0.92 s | | 0.98 s | |
| H-19 | 0.71 s | | 0.73 s | |
| H-21 | 1.02 d (7.2) | 2.61, 2.38, 1.08, 1.68, 1.32, 1.51, 0.60 | 1.03 d (7.0) | 2.50, 2.41, 1.13, 1.61, 1.36, 1.57, 0.61 |
| H-22 | 6.04 d (15) | 7.49, 5.96, 1.84 | 2.67 dd (16.5, 8.8) | 2.51, 4.76, 5.16, 1.67 |
| | | | 2.51 dd (16.5, 3.2) | 2.67, 4.76, 5.16, 1.67 |
| H-23 | 7.49 dd (11.7, 15) | 6.04, 5.96, 1.84 | 4.76 td (8.8, 3.2) | 5.16, 1.67, 2.67, 2.51 |
| H-24 | 5.96 d (11.7) | 7.49, 6.04, 1.84 | 5.16 dm (8.8) | 4.76, 2.67, 2.51, 1.67 |
| H-26 | 1.82 s[b] | | 1.67 d (1.2)[b] | 5.16, 4.76, 2.67, 2.51 |
| H-27 | 1.84 s[b] | 5.96, 7.49, 6.04 | 1.69 d (1.2)[b] | |
| H-28 | 0.92 s | | 0.97 s | |
| H-29 | 0.76 s | | 0.79 s | |
| H-30 | 4.35 d (10.5) | 4.20 | 4.38 d (10.5) | 4.21 |
| | 4.20 d (10.5) | 4.35 | 4.21 d (10.5) | 4.38 |

[a]Multiplicity (*J*, Hz).
[b]Assignments interchangeable within column.




**4** R=rha-glc-O, R′=CH=CH-CH=$CMe_2$
**5** R=rha-glc-O, R′=$CH_2$CH(OH)CH=$CMe_2$
**6** R=glc-O, R′=$CH_2$CH(OH)CH=$CMe_2$
**7** R=glc-O, R′=CH=CH-CH=$CMe_2$

sugars detected following acid-catalyzed hydrolysis of the purified saponin complex. The glucose C-6 resonances at 67 ppm, the fabms of **4** which showed negative ions at *m/z* 777 $[M-H]^-$, 631 $[M-(C_6H_{10}O_4)]^-$, and 469 $[M-(C_{12}H_{20}O_9)]^-$, and $^1H$ nmr signals at δ 4.7 (bs) and 4.3 (d, $J=7.8$ Hz) suggested the disaccharide unit, α-L-rhamnose-(1↦6)-β-D-glucose. (The absolute configurations of the sugars were chosen in keeping with those most commonly encountered among plant glycosides.) This conclusion was verified in part by the 500-MHz $^1H$ nmr of progouanogenin B [**6**], a product of incomplete enzymatic hydrolysis. Signals for the glycosidic protons of **6**, at δ 4.31 (d, $J=8.5$ Hz), 3.83 (dd, $J=12$, 2 Hz), 3.65 (dd, $J=12$, 5.5 Hz), 3.32 (t, $J=8.5$ Hz), 3.27 (t, $J=8.5$), 3.23 (ddd, $J=8.5$, 5.5, 2 Hz), and 3.18 (t, $J=8.5$ Hz) are indicative of a β-glucose. Thus **6** is 3-*O*-β-D-glucopyranosyl gouanogenin B. An additional product of incomplete enzymatic hydrolysis, isolated in trace amounts, exhibited a $^1H$ nmr consistent with formulation as 3-*O*-β-D-glucopyranosyl gouanogenin A [**7**].

The stereochemical assignments shown for compounds **1**, **3**, **4**, **5**, **6**, and **7**, typical of dammarane-type triterpenes, were fully validated by results of COSY and NOESY experiments on progouanogenin B [**6**]; the data are summarized in Table 3. Of particular import are nOe crossover peaks observed for Me-29–Me-19, Me-19–Me-18, Me-18–H-13 and for H-3–H-5, H-5–H-9, H-9–H-30β, which define, respectively, the "top" and "bottom" of the trans-fused tricyclic system (Figure 1). Additional crossover peaks involving H-1′–H-3 and H-1′–Me-28 reflect the relative configuration of glucose and aglycone (20,21). Significant correlations involving H-17 and Me-21 were also observed; however, no stereochemical conclusions are possible since the conformation of the side chain is not known, a point currently under investigation.

It is interesting to note that 16,17-*seco*-dammaranes (and precursors thereto) are known principally among the Rhamnaceae (22), suggesting a common oxidative process for this family. The novel structural characteristic of gouanosides A and B, the "shifted methyl group," may be an associated feature.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—A Varian XL-300 spectrometer was employed for routine $^1H$- and $^{13}C$-nmr measurements; carbon substitution was determined by either APT or DEPT experiments. All spectra were referenced to the solvent used and chemical shifts are reported in ppm downfield from TMS. The 2D experiments, HMQC, HMBC, TOCSY, PCOSY, and NOESY, were carried out at 500 or 600 MHz on Varian Unity systems and the data processed on a Sun SPARC 1 work station. Low resolution eims was obtained at 70 eV on a Hewlett-Packard 5988A Mass Spectrometer; samples were introduced by direct insertion using a heated probe. Fabms and high resolution fabms spectra were obtained on a VG 70 SE instrument fitted with a standard fab source. Samples were dispersed in thioglycerol and bombarded with a beam of Xe atoms with an acceleration of 8 kV; no attempts were made to remove extraneous salt. Ir spectra were taken as KBr pellets on a Perkin-Elmer 1600 Series FTIR spectrometer, and uv spectra were measured on MeOH solution using a Hitachi U-3110 spectrophotometer.

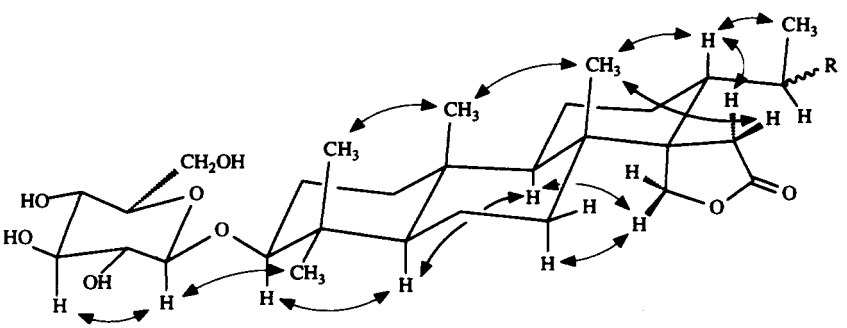



FIGURE 1. Important nOe's in progouanogenin B [**6**] (R=-$COCH_2CHOHCH{=}CMe_2$).

TABLE 3. $^1$H-nmr Data for Progouanogenin B [6] (500 MHz, $CD_3OD$).

| Proton[a] | δ ppm multiplicity (*J*, Hz) | COSY[b] | NOESY[b] |
|---|---|---|---|
| H-1ax | 0.98 m | 1.72, 1.66 | 0.74 |
| H-1eq | 1.72 m | 0.98 | |
| H-2ax | 1.66 m | 0.98, 1.95, 3.17 | |
| H-2eq | 1.95 m | 1.66 | |
| H-3 | 3.17 dd (11, 5) | 1.66 | 0.80, 1.06 |
| H-5 | 0.80 dd (12, 2) | 1.48 | 0.74, 3.17 |
| H-6ax | 1.48 m | 0.80 | |
| H-6eq | 1.52 m | | |
| H-7ax | 1.44 m | | 4.46 |
| H-7eq | 1.55 m | | 2.67 |
| H-9 | 0.74 dd (12, 2.5) | 1.42 | 0.90, 0.98, 4.46 |
| H-11eq | 1.59 m | | |
| H-11ax | 1.42 m | 0.74, 1.26 | |
| H-12ax | 1.26 m | 1.42, 1.60, 2.45 | 4.33 |
| H-12eq | 1.60 m | 1.26 | |
| H-13 | 2.45 dbt (13, 3) | 1.26 | 1.02, 1.04, 2.25 |
| H-15α | 2.25 d (19) | 2.67 | 2.45, 2.56 |
| H-15β | 2.67 d (19) | 2.25 | 1.04, 1.55 |
| H-17 | 2.56 qd (7.0, 3) | 1.02 | 2.25, 4.33 |
| H-18 | 1.04 s | | 0.87, 2.45, 2.67 |
| H-19 | 0.87 s | | 0.84, 1.04 |
| H-21 | 1.02 d (7.0) | 2.56 | 2.45, 4.33 |
| H-22 | 2.72 dd (15.5, 8.5) | 2.52, 4.77 | 5.17 |
| | 2.52 dd (15.5, 5.5) | 2.72, 4.77 | 5.17 |
| H-23 | 4.77 dt (8.5, 5.5) | 2.52, 2.72, 5.17 | 1.68 |
| H-24 | 5.17 dm (8.5) | 1.68, 1.70, 4.77 | 1.70, 2.52, 2.72 |
| H-26 | 1.70 d (1.5) | 5.17 | 5.17 |
| H-27 | 1.68 d (1.5) | 5.17 | 4.77 |
| H-28 | 1.06 s | | 0.84, 3.17, 4.31 |
| H-29 | 0.84 s | | 0.87, 1.06 |
| H-30α | 4.33 d (11) | 4.46 | 1.02, 1.26, 2.56 |
| H-30β | 4.46 d (11) | 4.33 | 0.74, 1.44 |
| H-1′ | 4.31 d (8.5) | 3.18 | 1.06, 3.23, 3.32 |
| H-2′ | 3.18 t (8.5) | 3.32, 4.31 | |
| H-3′ | 3.32 t (8.5) | 3.18 | 4.31 |
| H-4′ | 3.27 t (8.5) | | 3.65, 3.83 |
| H-5′ | 3.23 ddd (8.5, 5.5, 2.0) | 3.65 | 4.31 |
| H-6′ | 3.83 dd (12, 2.0) | 3.65 | 3.27 |
| | 3.65 dd (12, 5.5) | 3.23, 3.83 | 3.27 |

[a]Assignments based in part on HMQC ahd HMBC experiments.
[b]Only the prominent cross peaks are listed.

Tlc analyses were performed on Kieselgel 60 F254 (Merck); compounds were visualized by spraying either with Liebermann-Burchard reagent (9) or a vanillin spray reagent (8) and then charring. The specific detection of saponins was accomplished by the blood-gelatin tlc overlay method (9). Silica gel, Merck 60 Å, 230–400 mesh ASTM (Aldrich Chemical Co.) was used for flash chromatography. Hplc separations were carried out using a Perkin-Elmer Series 3B Liquid Chromatograph equipped with a variable wavelength uv detector. A Whatman Partisil 5 column, (250 mm × 0.46 mm i.d.) was employed for analytical hplc and a Partisil Megabore column (250 mm × 20 mm i.d.) for preparative separations.

PLANT MATERIAL.—The population of *G. lupuloides* used in all of the isolation work was collected in Claredon, Jamaica by Mr. Leonard Henry in May, 1988. A voucher specimen is located at the herbarium of the Missouri Botanical Garden, St. Louis, Missouri. The woody stems were air-dried and stored at room temperature until used.

ISOLATION OF SAPONINS.—Finely ground plant stems were exhaustively extracted with MeOH at room temperature resulting in a light green solution. After washing with hexanes and stripping all MeOH under reduced pressure, the residue was partitioned between *n*-BuOH and $H_2O$. The crude saponin complex, obtained on evaporation of the organic layer, accounted for about 5% of the original weight of plant material. Solutions caused hemolysis of red blood cells (ditch method). Purification was accomplished by flash chromatography, eluting the $CHCl_3$-MeOH (4:1→2:3) and finally stripping with MeOH. Fractions containing material $R_f$ ca. 0.2 [$CHCl_3$-MeOH (4:1)] tested positive to Lieberman-Burchard and vanillin spray reagents and caused hemolysis of red-blood cells (overlay method). These fractions were pooled, furnishing purified saponin complex as a light tan semi-solid, ca. 0.4 g per g of crude saponin.

ACIDIC HYDROLYSIS OF SAPONIN MIXTURE.—A solution of the purified saponin complex in MeOH was mixed with an equal volume of 10 N HCl and heated at 85° for 20 min. After cooling and diluting with $H_2O$, the mixture was extracted exhaustively with $Et_2O$. The $Et_2O$ layers were combined, washed, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. Tlc analysis of the residue [EtOAc-hexanes (1:1)] showed a single major product, $R_f$ 0.75, and flash chromatography furnished tlc homogeneous gouanogenin A [**1**] (ca. 10 mg per 100 mg saponin complex): mp 195–200°; $^1$H nmr (300 MHz, $CDCl_3$) δ 7.53 (dd, $J$ = 11.7, 14.7 Hz, 1H), 6.07 (d, $J$ = 14.7 Hz, 1H), 5.98 (d, $J$ = 11.7 Hz, 1H), 4.39 (d, $J$ = 10 Hz, 1H), 4.23 (d, $J$ = 10 Hz, 1H), 3.18 (dd, $J$ = 11.5, 5 Hz, 1H), 2.64 (qd, $J$ = 7.2, 4 Hz, 1H), 2.54 (d, $J$ = 19 Hz, 1H), 2.38 (dbt, $J$ = 13.2, 4 Hz, 1H), 2.06 (d, $J$ = 19 Hz, 1H), 1.90 (s, 3H), 1.88 (s, 3H), 1.05 (d, $J$ = 7.2 Hz, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.86 (s, 3H), 0.76 (s, 3H); $^{13}$C nmr (75 MHz, $CDCl_3$) see Table 1; uv λ max 295; ir ν max 3500, 1772, 1685, 1654, 1628 $cm^{-1}$; eims *m/z* (rel. abundance) 470 (1.4), 455 (3.7), 189 (2.5), 147 (2.1), 139 (4.8), 138 (25), 137 (7.5), 135 (4.9), 133 (2.8), 123 (13), 110 (8), 109 (100), 107 (5.6), 105 (4.5), 95 (7.1), 93 (5.9), 91 (4.6), 81 (19.9), 79 (9.5), 69 (5.2), 67 (4.7), 55 (5.3), 43 (4.7); fabms *m/z* $[M+H]^+$ 471; hrfabms found 471.3457 (calcd for $C_{30}H_{47}O_5$, 47.3474).

Solutions of purified gouanogenin A deteriorated readily. Even dried samples exhibited several more polar components (tlc) after only several weeks refrigeration.

The aqueous layer from an acidic hydrolysis of the saponin mixture in dioxane/$H_2O$ was neutralized with an ion-exchange resin (Rexyn I-300) and concentrated in vacuo. Tlc analysis of the residue [MeCOMe-$H_2O$ (9:1)] showed the presence of only two sugars, these having the same $R_f$ and exhibiting the same color with anisaldehyde spray reagent (8) as authentic samples of D-glucose and L-rhamnose.

ENZYMATIC HYDROLYSIS OF SAPONIN MIXTURE.—A solution containing approximately equal weights of purified saponin complex and naringinase (Sigma Chemical Company) in 0.1 M acetate buffer, pH 5.6 (4–5 ml per 100 mg saponin) was incubated at 37° for 4–7 days. The reaction was followed by tlc, and the appearance of less polar products was noted after 24 h. The reaction was interrupted by diluting the mixture with $H_2O$ and extracting exhaustively with $CH_2Cl_2$. The combined organic layers were washed and passed over anhydrous $Na_2SO_4$, and the solvent was removed under reduced pressure. Tlc analysis of the residue [EtOAc-hexanes (1:1)] showed a trace of gouanogenin A ($R_f$ 0.75) and only one other major non-polar component ($R_f$ 0.70). Flash chromatography using a solvent gradient of $CHCl_3$-MeOH (35:1→4:1) furnished chromatographically homogeneous gouanogenin B [**3**] as a nearly colorless glass (yields variable from 5 to 25 mg per 100 mg saponin): $^1$H nmr (300 MHz, $CDCl_3$) δ 5.15 (bd, $J$ = 8.8 Hz, 1H), 4.76 (td, $J$ = 8.8, 3 Hz, 1H), 4.37 (d, $J$ = 10.5 Hz, 1H), 4.22 (d, $J$ = 10.5 Hz, 1H), 3.17 (dd, $J$ = 11, 5 Hz, 1H), 2.68 (dd, $J$ = 16, 9 Hz, 1H), 2.60 (d, $J$ = 19 Hz, 1H), 2.51 (dd, $J$ = 16, 3 Hz, 1H), 2.50 (qd, $J$ = 7, 3 Hz, 1H), 2.43 (vbd, $J$ = 14 Hz, 1H), 2.12 (d, $J$ = 19 Hz, 1H), 1.69 (s, 3H), 1.67 (s, 3H), 1.04 (d, $J$ = 7 Hz, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.81 (s, 3H), 0.76 (s, 3H); $^{13}$C nmr (75 MHz, $CDCl_3$) see Table 1.

Treatment of gouanogenin B with base produced gouanogenin A (tlc analysis), and solutions of **3** in $CDCl_3$ exhibited $^1$H-nmr signals characteristic of **1** after standing at room temperature for several days.

Products of incomplete enzymatic hydrolysis were also evident as minor polar components in the crude reaction mixture. These were separated by flash chromatography furnishing homogeneous progouanogenin B [**6**] as a colorless solid: $R_f$ 0.35 [$CHCl_3$-MeOH (4:1)]; $^1$H nmr (300 MHz, $CD_3CN/D_2O$) δ 5.08 (bd, $J$ = 8.5 Hz, 1H), 4.64 (m, 1H), 4.39 (d, $J$ = 11 Hz, 1H), 4.29 (d, $J$ = 8.5 Hz, 1H), 4.23 (d, $J$ = 11 Hz, 1H), 3.69 (bd, $J$ = 12 Hz, 1H), 3.56 (dd, $J$ = 12, 4 Hz, 1H), 3.16–3.33 (m, 3H), 3.08 (bt, $J$ = 8.5 Hz, 1H), 3.08 (m, 1H), 2.65 (dd, $J$ = 15, 8.5 Hz, 1H), 2.60 (d, $J$ = 19 Hz, 1H), 2.52 (dd, $J$ = 15, 5 Hz, 1H), 2.43 (m, 1H), 2.27 (vbd, $J$ = 12 Hz, 1H), 2.14 (d, $J$ = 19 Hz, 1H), 1.62 (bs, 3H), 1.58 (bs, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.90 (d, $J$ = 7 Hz, 3H), 0.77 (s, 3H), 0.73 (s, 3H); $^{13}$C nmr (75 MHz, $CD_3CN/D_2O$) see Table 1.

Fractions preceding those containing **6** contained progouanogenin A [**7**]: $^1$H nmr (300 MHz, $CD_3CN/D_2O$) δ 7.47 (dd, $J$ = 15, 11 Hz), 6.14 (d, $J$ = 15 Hz), 6.02 (d, $J$ = 11 Hz), 4.39 (d, $J$ = 11 Hz),

4.29 (d, $J$ = 8 Hz), 4.27 (m), 4.25 (d, $J$ = 11 Hz), 2.54 (d, $J$ = 19 Hz), 2.04 (d, $J$ = 19 Hz), 1.87 (s), 1.85 (s), 0.96 (s), 0.94 (s), 0.91 (s), 0.79 (s), 0.75 (s).

ISOLATION OF GOUANOSIDES A AND B.—Hplc analysis [MeCN-$H_2O$ (95:5) λ detect 207 nm] of the purified sponin complex showed it to be a mixture, the two principal components of which were preparatively separated. Each was obtained as an amorphous chromatographically homogeneous solid, following drying at room temperature under reduced pressure.

*Gouanoside A* [**4**].—Rt 6.5 min, flow 1.5 ml/min; $^1$H nmr (300 MHz, $CD_3CN$) δ 7.47 (dd, $J$ = 15, 11.5 Hz, 1H), 6.15 (d, $J$ = 15 Hz, 1H), 6.03 (d, $J$ = 11.5 Hz, 1H), 4.68 (bs, $w_{1/2h}$ 3 Hz, 1H), 4.39 (d, $J$ = 10.7 Hz, 1H), 4.26 (vbd, 2H), 3.9–3.5, 3.39–3.21, 3.15–3.00 (cm, 2H), 2.71 (qd, $J$ = 6.8, 4.6 Hz, 1H), 2.54 (d, $J$ = 19 Hz, 1H), 2.32 (dbt, $J$ = 12, 3.5 Hz, 1H), 2.05 (d, $J$ = 19 Hz, 1H), 1.88 (bs, 3H), 1.86 (bs, 3H), 1.17 (d, $J$ = 7.0 Hz, 3H), 0.97 (d, $J$ = 7.0 Hz, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.79 (s, 3H), 0.77 (s, 3H); $^{13}$C nmr (75 MHz, $CD_3CN$) see Table 1; fabms *m/z* $[M-H]^-$ 777, $[M-C_6H_{10}O_4]^-$ 631, $[M-C_{12}H_{20}O_9]^-$ 469.

*Gouanoside B* [**5**].—Rt 7.4 min, flow 1.5 ml/min; $^1$H-nmr (300 MHz, $CD_3CN$) δ 5.14 (bd, $J$ = 8.7 Hz, 1H), 4.74 (bs, $w_{1/2h}$ 3 Hz, 1H), 4.71 (m, 1H), 4.42 (d, $J$ = 10 Hz, 1H), 4.31 (d, $J$ = 7.8 Hz, 1H), 4.28 (d, $J$ = 10 Hz, 1H), 3.90 (bd, $J$ = 11 Hz, 1H), 3.83 (m, 1H), 3.8–3.2 (cm), 3.12 (dd, $J$ = 11, 4 Hz, 1H), 2.71 (dd, $J$ = 15, 7.8 Hz), 2.60 (d, $J$ = 19 Hz), 2.58 (m), 2.50 (dd, $J$ = 15, 5 Hz, 1H), 2.43 (m, 1H), 2.23 (d, $J$ = 19 Hz, 1H), 1.62 (bs, 3H), 1.60 (bs, 3H), 1.02 (d, $J$ = 7 Hz, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.96 (d, $J$ = 7 Hz, 3H), 0.81 (s, 3H), 0.78 (s, 3H); $^{13}$C nmr (75 MHz, $CD_3CN$) see Table 1.

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