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ABRUSOSIDES A–D, FOUR NOVEL SWEET-TASTING TRITERPENE GLYCOSIDES FROM THE LEAVES OF *ABRUS PRECATORIUS*[1]

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ABSTRACT.—In addition to abrusoside A [**1**], abrusosides B [**2**], C [**3**], and D [**4**], three further sweet glycosides based on the novel cycloartane-type aglycone, abrusogenin [**5**], were isolated from an *n*-BuOH-soluble extract of the leaves of *Abrus precatorius*. Using a combination of spectral methods, the structures of compounds **1–4** were assigned, respectively, as the 3-*O*-β-D-glucopyranosyl, the 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-6-methylglucuronopyranosyl, the 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl, and the 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl derivatives of compound **5**. After it established that compounds **1–4** were neither acutely toxic with mice nor mutagenic with *Salmonella typhimurium* strain TM677, they were found by a human taste panel to exhibit sweetness potencies in the range 30–100 times greater than sucrose.

Abrus precatorius L. (Fabaceae) is a vine originally native to India that is now commonly distributed throughout tropical and subtropical regions of the world (2). Poisoning by the seeds of this species is well documented (2–4), with abrin, the glycoprotein responsible, being regarded as one of the most potent of all known toxins (5). Despite the known toxicity of *A. precatorius* seeds, its roots have been used since at least the early 19th century as a substitute for licorice root, the source of the sweet oleanane-type triterpene glycoside, glycyrrhizin (6–9). However, according to some literature sources, the leaves of *A. precatorius* are sweeter than the roots (6,7,10), and they have been described by Inglett and May (11) as equivalent in sweetness potency to sucrose. In West Tropical Africa, *A. precatorius* leaves have been employed to sweeten foods and certain medicines used for stomach complaints (9,12). The leaves are casually chewed and the vine sometimes sold as a masticatory in Curaçao (9). Previous workers have claimed that glycyrrhizin is the sweet principle of *A. precatorius* leaves (7,10, 12–14), and optimum greenhouse conditions have been developed for the cultivation and propagation of *A. precatorius* for the production of glycyrrhizin (15). Other reported constituents of *A. precatorius* leaves include the triterpenes, abrusgenic acid, abruslactone A, and methyl abrusgenate (16–18), and the nitrogen-containing compounds precatorine and trigonelline (19).

In a preliminary report we have provided brief details of the isolation and characterization of abrusoside A [**1**], a sweet *A. precatorius* leaf constituent obtained after we were initially unable to detect the presence of glycyrrhizin in the particular plant sample investigated (20). Abrusogenin [**5**], the aglycone obtained on the acid hydrolysis of this sweet compound, was found by the interpretation of spectroscopic data to be a cycloartane derivative with a δ-lactone ring, and its structure was confirmed by single-crystal X-ray crystallography performed on its methyl ester **6** (20). In the present communication, full details of the characterization of abrusoside A [**1**] are provided, and three further sweet constituents of *A. precatorius* leaves, abrusosides B [**2**], C [**3**], and D [**4**], have been structurally determined. All four sweet principles **1–4** have been subjected to preliminary safety and sensory evaluations.

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[1]Paper No. XXI in the series, "Potential Sweetening Agents of Plant Origin." For part XX, see Hussain *et al.* (1).

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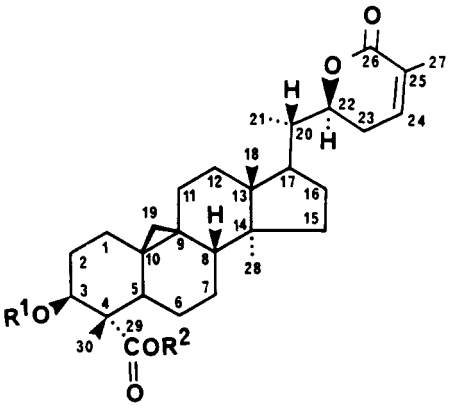

	R^1	R^2
1	β-D-glc	H
2	β-D-glcA-6-CH_3^2-β-D-glc	H
3	β-D-glc^2-β-D-glc	H
4	β-D-glcA^2-β-D-glc	H
5	H	H
6	Ac	H
7	H	Me

RESULTS AND DISCUSSION

In an initial study, crude extracts of the leaves of *A. precatorius* were found to be devoid of glycyrrhizin when compared by tlc with an authentic sample of this triterpene glycoside. Because it was then suspected that the perceived sweetness of *A. precatorius* leaves might be due to glycyrrhizin occurring in low concentrations, a fractionation procedure was designed to concentrate this compound (if present) in *n*-BuOH extract III (see Experimental). Although the tlc analysis of this purified extract did not reveal the presence of glycyrrhizin, four major compounds were detected that gave a similar chromotropic response. On purification and spectroscopic characterization, these four new compounds, which we have called abrusosides A [**1**], B [**2**], C [**3**], and D [**4**], were found to be based upon a novel cycloartane-type aglycone, abrusogenin [**5**]. Owing to their general structural similarity to glycyrrhizin, compounds **1–4** were assessed for sweetness by a small human taste panel, after preliminary safety studies. Compounds **1–4** were found to be sweet, while their aglycone **5** and two derivatives, abrusogenin methyl ester [**6**] and abrusogenin 3-monoacetate [**7**], did not exhibit any sweet taste.

Only a brief outline of the spectroscopic evidence for the structural assignment of abrusogenin [**5**] has been published thus far (20), and further details are provided in the following paragraphs. Assignments of the ^{1}H- and ^{13}C-nmr spectra of abrusogenin [**5**] were facilitated by performing ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr experiments. Thus, compound **5** was suspected to contain a cyclopropyl ring system, as a result of the observation of two doublets (1H each) appearing at δ 0.61 and 0.39 in its ^{1}H-nmr spectrum and the characteristic resonances seen in its ^{13}C-nmr spectrum at δ 29.93 (t, C-19), 25.09 (s, C-10), and 19.95 (s, C-9) (21–24). The prominent mass spectral fragment peak observed for **5** at *m/z* 314.2245 ($C_{21}H_{30}O_2$) is consistent with this compound containing a 9,19-cyclopropyl-lanostane ring system (25). The connectivity of the cyclopropane ring to carbons C-9 and C-10 of abrusogenin [**5**] was confirmed by ap-

glc = glucopyranosyl; glcA = glucuronopyranosyl

plication of the selective INEPT nmr technique (26). Irradiation of the H-19 proton at δ 0.61 ($^3J_{CH}$ = 6 Hz) selectively enhanced carbons C-1, C-5, C-8, C-9, C-10, and C-11, which are two and three bonds distant from this proton.

Spectral evidence was obtained for the presence of an α,β-unsaturated δ-lactone ring in the molecule of compound **5**, including the ir absorption band at 1707 cm^{-1} and the carbonyl resonance at δ 167.13 in the ^{13}C-nmr spectrum (27). An olefinic chemical shift that appeared at δ 6.63 in the ^{1}H-nmr spectrum of **5** was assigned to a proton β to this carbonyl group, while a vinylic methyl group observed at δ 1.91 was placed at the carbon α to the lactone carbonyl group. Other proton placements were made by spectral data comparison with substituents in the α,β-unsaturated δ-lactone ring of the model compound, schisanlactone B (27). Confirmation of the relative arrangements of the lactone ring substituents of abrusogenin [**5**] was made by the application of the selective INEPT technique, with irradiation of H-22 (δ 4.50), H-23 (δ (2.58), and H-24 (δ 6.63) ($^3J_{CH}$ = 6 Hz) leading, respectively, to enhancements of C-17, C-20, C-21, C-23, and C-24; C-20, C-22, C-24, C-25, C-26, and C-27; and C-22, C-23, C-26, and C-27. Analogous irradiation of Me-27 (δ 1.91, $^3J_{CH}$ = 8 Hz) selectively enhanced the carbons at positions 24, 25, and 26 of compound **5**. The stereochemistry of position 22 in the abrusogenin [**5**] molecule was proposed as *S* on the basis of the appearance of a signal in the ^{1}H-nmr spectrum of H-22 as a doublet of doublets (δ 4.50). Among model compounds with a δ-lactone ring, similar multiplicity has been observed for 22*S* stereochemistry, whereas a doublet of triplets has been associated for compounds with 22*R* stereochemistry (28–30).

A carbonyl signal (δ 180.19) appearing in the ^{13}C-nmr spectrum of abrusogenin [**5**] resulted from the presence of a carboxylic acid unit, a functionality that was confirmed by the conversion of **5** to its methyl ester **6**. The position of attachment of this group was proposed as C-4 by data comparison with the ^{13}C-nmr spectra of cycloartenol (22) and mollic acid 3-β-D-glucoside (31) and then verified by means of a selective INEPT nmr experiment. Thus, when H-3 (δ 4.09) was irradiated ($^3J_{CH}$ = 6 Hz), carbons at positions 2, 4, 29, and 30 were selectively enhanced. The stereochemistry of this acidic substituent was assigned as α by comparison of the C-4 ^{13}C-nmr chemical shift of **5** (δ 54.57) with chemical shift ranges exhibited by model compounds with C-4 carbon atoms that bear either α- (δ 53–55) or β- (δ 49–50) substituted carboxylic acids (32,33). Compound **5** was converted to a monoacetate [**6**] under normal conditions of acetylation, and the stereochemistry of the C-3-affixed hydroxy group was determined as β by observation of the coupling constants (J = 11.1 and 4.3 Hz) of H-3α at δ 4.09 (30,34). In this manner, the structure of abrusogenin [**5**] was proposed as (20*S*,22*S*)-3β,22-dihydroxy-9,19-cyclolanost-24-en-26,29-dioic acid δ-lactone. Subsequently, the structure and stereochemistry of **5** were confirmed by application of X-ray crystallography on a sample of abrusogenin methyl ether [**6**] (20).

The structure elucidation of abrusosides A [**1**], B [**2**], C [**3**], and D [**4**] was accomplished with the particular aid of two modern spectroscopic techniques, namely, high resolution fabms and the selective INEPT nmr technique. Also, comparison of ^{13}C-nmr chemical shift data obtained in this investigation with published data for the saccharide moieties of other naturally occurring glycosides proved highly useful. In all cases, assignments of ^{1}H- and ^{13}C-nmr spectra were made with the use of supporting ^{1}H-^{13}C HETCOR nmr experiments. Abrusogenin [**5**] and one or more sugars were obtained on acid hydrolysis of the sweet glycosides **1–4**, with the identities of the hydrolyzed sugars being checked for each heteroside by gc-ms and tlc using authentic substances.

The molecular formula of abrusoside A [**1**] was established as $C_{36}H_{54}O_{10}$ by high resolution fabms, and comparison of its ^{13}C-nmr spectrum with that of its aglycone [**5**]

indicated that sugar substitution occurred at the C-3 position. This was confirmed using the selective INEPT nmr method on the intact glycoside (26,35), by irradiating the anomeric proton of **1** at δ 5.14 ($^3J_{CH}$ = 6 Hz), which selectively enhanced C-3 at δ 85.31, and by irradiating the H-3 proton (δ 4.72) in a similar manner, which enhanced the anomeric carbon (δ 105.50). The sugar and its configuration were established as β-D-glucopyranose with reference to ^{13}C-nmr chemical shift data published for various sugars in ginsenosides and other glycosides (36–38). The structure of abrusoside A [**1**] was therefore determined as abrusogenin-3-*O*-β-D-glucopyranoside.

Abrusoside B [**2**], $C_{43}H_{64}O_{16}$, afforded D-glucose and D-glucuronic acid methyl ester on hydrolysis. When the ^{13}C-nmr spectrum of **2** was considered against analogous data obtained for compounds **1** and **5**, it was apparent that both sugars in the molecule of **2** were affixed to C-3. The saccharide linkage in abrusoside B [**2**] was determined as β-D-glucuronic acid-6′-methyl ester-(2↦1)-β-D-glucose by comparison with ^{13}C-nmr chemical shifts of model glycosides and because of the observation of anomeric ^{1}H-nmr (J = 7.6 Hz for H-1″ and W½ = 7.1 Hz for H-1′) and ^{13}C-nmr (δ 106.35 for C-1″ and δ 102.47 for C-1′) chemical shifts for compound **2** (39,40). The selective INEPT technique has previously been used to determine sugar linkages in oligosaccharides (41) and was utilized in this study to investigate the order of saccharide substitution in abrusoside B [**2**]. When the H-1″ proton at δ 5.37 ($^3J_{CH}$ = 6 Hz) was irradiated, C-2′ (of glucose) (δ 84.05) was selectively enhanced, while irradiation of the 3-H proton of the aglycone (δ 4.87, $^3J_{CH}$ = 6 Hz) enhanced C-1′ of glucose. Therefore, the structure of abrusoside B [**2**] was determined as abrusogenin-3-*O*-β-D-glucopyranosyl)-(1↦2)-β-D-glucuronopyranoside-6′-methyl ester.

Abrusoside C [**3**] produced only one sugar upon acid hydrolysis, namely D-glucose, although it was apparent from its elemental formula of $C_{42}H_{64}O_{15}$, obtained using high resolution fabms, that two glucose residues were present in this sweet molecule. The sugar unit of **3** was established as β-D-glucose-(2↦1)-β-D-glucose (sophorose) by comparison of its ^{13}C-nmr chemical shifts with those of model glycosides and analysis of the coupling constants (J = 7.7 Hz for H-1″ and J = 7.6 Hz for H-1′) and chemical shifts (δ 105.80 for C-1″ and δ 103.63 for C-1′) of anomeric sites in its ^{1}H- and ^{13}C-nmr spectra (36,42). It is of interest that stevioside, the major sweet *ent*-kaurene glycoside of *Stevia rebaudiana* (Bertoni) Bertoni leaves, also contains a sophorosyl unit (36). The position of the sophorosyl attachment and its linkage were confirmed with selective INEPT experiments, wherein irradiation of H-1′ at δ 5.23 ($^3J_{CH}$ = 6 Hz) selectively enhanced C-3 (δ 84.96) and analogous irradiation of H-1″ at δ 5.15 enhanced C-2′ (δ 83.88), the position of attachment of the second glucose residue. The chemical shifts of C-1′ (δ 103.63) and C-3 (δ 84.96) were confirmed by irradiating H-3 (δ 4.70, $^3J_{CH}$ = 6 Hz) and Me-30 (δ 1.66, $^3J_{CH}$ = 8 Hz), respectively. Thus, the structure of abrusoside C was determined as abrusogenin-3-*O*-β-D-glucopyranosyl-(1↦2)-β-D-glucopyranoside [**3**].

The most polar and most abundant of the sweet compounds obtained in this investigation, abrusoside D [**4**], was found to have a molecular formula of $C_{42}H_{62}O_{16}$ using high resolution fabms. On hydrolysis, both D-glucose and D-glucuronic acid were identified. When investigated by procedures similar to those applied to the other three sweet compounds **1–3** isolated, the position of sugar substitution in the aglycone of **4** was again established at the C-3 position, and the saccharide unit concerned was determined as β-D-glucuronic acid-(1↦2)-β-D-glucose on comparison of observed ^{13}C-nmr chemical shifts with those of model compounds and analysis of coupling constants and chemical shifts at the anomeric sites (J = 7.4 Hz and δ 105.77 for C-1″, and J = 7.2 Hz and δ 102.59 for C-1′) of compound **4** (36,39). In selective INEPT experiments, irradiation of H-1″ at δ 5.22 ($^3J_{CH}$ = 6 Hz), selectively enhanced C-2 (δ 83.08), and

similar irradiation of H-3 (δ 4.83) enhanced C-1′. The chemical shift of C-3 (δ 83.71) was confirmed by irradiation of the C-30 methyl protons (δ 1.70, $^3J_{CH}$ = 8 Hz). The structure of abrusoside D was therefore determined as abrusogenin-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranoside [**4**].

Prior to being assessed for sweetness, the initial MeOH/H_2O layer of *A. precatorius* leaves and abrusosides A [**1**], B [**2**], C [**3**], and D [**4**] were shown to be nontoxic in preliminary acute toxicity tests in mice (43–45) and in forward mutation assays utilizing *Salmonella typhimurium* strain TM677 (43–46). The sweetness intensities of abrusosides A [**1**], B [**2**], C [**3**], and D [**4**] were evaluated by a small taste panel (43,44) and were ranked as about 30, 100, 50, and 75 times sweeter than sucrose, respectively. Therefore, abrusosides A [**1**], B [**2**], C [**3**], and D [**4**], rather than glycyrrhizin, are responsible for the sweet taste of the leaves of the common subtropical species, *A. precatorius*, where they were found to occur in a total yield of 0.39% dry wt. These compounds did not appear to exhibit any unpleasant taste along with their sweet effect, and can easily be rendered H_2O-soluble by conversion to their ammonium salts. Abrusosides A [**1**], B [**2**], C [**3**], and D [**4**] do not contain an α,β-unsaturated keto group such as the 11-oxo-12,13-dehydro-group found in glycyrrhizin, to which has been attributed the latter's undesirable adrenocorticomimetic effects (47,48). Hence, although abrusosides A–D possess the same general order of sweetness potency as glycyrrhizin, these compounds would seem unlikely to exhibit the same type of toxic effects as the latter substance. Further studies are necessary to determine the feasibility of using either *A. precatorius* leaf extracts or purified abrusosides A–D commercially for the sweetening of foods, beverages, and medicines. For example, more extensive subacute and chronic toxicity tests in laboratory animals would have to be conducted to discern more fully the safety of these compounds for human consumption.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The uv spectra were obtained on a Beckman DU-7 spectrometer and ir spectra measured on a Nicolet MX-1 FT-ir (KBr) interferometer. ^{1}H-nmr spectra were recorded with TMS as internal standard, employing either a Nicolet NT-360 or a Varian XL-300 instrument (360 MHz or 300 MHz, respectively). Low- and high-resolution mass spectra were obtained with a Varian MAT 112S instrument operating 70 eV. Gc-ms was performed on a Finnigan 4510 instrument, using a DB-1 column, under conditions that have been described previously (1). Droplet counter-current chromatography (dccc) was performed on a Model-A instrument (Tokyo Rikakikai, Tokyo, Japan).

PLANT MATERIAL.—The leaves of *A. precatorius* were collected in Miami, Florida in February 1986 and identified by one of us (J.F.M.). Voucher specimens documenting these collections are deposited in the Herbarium of the Field Museum of Natural History, Chicago, Illinois.

EXTRACTION AND FRACTIONATION.—The air-dried plant material (600 g) was extracted with MeOH-H_2O (4:1) and gave 130 g of a dried MeOH/H_2O extract on removal of solvent in vacuo. A portion of this residue (120 g) was partitioned between H_2O (3 liters) and Et_2O (4 × 1 liter), which yielded 22.0 g of a dried Et_2O extract that was not investigated further. The aqueous extract (H_2O extract I) was adjusted to pH 3 with 1 N HCl and then partitioned with H_2O-saturated *n*-BuOH (3 × 1 liter), to afford *n*-BuOH extract I and a further H_2O layer (H_2O extract II, 49 g on removal of solvent). *n*-BuOH extract I was adjusted to pH 10 with 1 N NH_4OH and washed with H_2O (3 × 1 liter) to produce *n*-BuOH extract II (14 g on drying) and H_2O extract III. The latter was acidified to pH 3 and extracted with *n*-BuOH (3 × 1 liter) to generate, on removal of the solvents, *n*-BuOH extract III (40 g) and H_2O extract IV (12 g). Glycyrrhizin (Aldrich Chemical Co., Milwaukee, Wisconsin) was not detected by tlc in either the initial MeOH/H_2O extract or *n*-BuOH extract III.

When MeOH (100 ml) was added to *n*-BuOH extract III (40 g), an MeOH-insoluble precipitate (15 g) was formed, which was dried and later used for the isolation of abrusoside D [**4**]. The dried mother liquor (25 g) was purified by gel filtration by dissolving in MeOH (50 ml), with a 5-ml portion applied to the top of a glass column (1.9 cm × 50 cm) packed with Sephadex® LH-20 (100 g) in MeOH. On elution with

MeOH, a total of 300 fractions (100 drops each) were collected and monitored by tlc on Si gel G plates using $CHCl_3$-MeOH-H_2O (65:35:10) (lower phase, solvent 1) as the developing solvent. Fractions showing similar tlc profiles were pooled to give five combined fractions, inclusive of fractions 28–59 which appeared as a series of violet zones on tlc plates when visualized with vanillin/H_2SO_4 spray reagent. This procedure was repeated an additional nine times to afford a total of 10 g of dried residue.

ISOLATION OF ABRUSOSIDES A [**1**], B [**2**], AND C [**3**].—Fractions 28–59 (10 g) were further fractionated by cc by dissolution in 20 ml of solvent 1 and impregnation of solute on Si gel (20 g). The impregnated Si gel was applied to the top of a glass column (5 cm × 120 cm, Pirkle® preparative column, Anspec, Ann Arbor, Michigan) packed with a slurry of Si gel (800 g, 230–400 mesh) in $CHCl_3$-MeOH (10:1). The column was eluted under low pressure at a flow rate of 4.5 ml/min, using mixtures of $CHCl_3$/MeOH of increasing polarity. Altogether, 45 fractions (600 ml each) were collected and were monitored by tlc on Si gel G plates using solvent 1 and $CHCl_3$-MeOH-H_2O (6:2:1) (lower layer, solvent 2). Fractions showing similar tlc profiles were pooled to give 11 combined fractions. Fraction 10 (0.52 g), which was eluted with $CHCl_3$-MeOH (100:15), was purified by repeated recrystallization in MeOH to afford abrusoside A [**1**] (180 mg, 0.033% dry wt) as needle-shaped crystals. This isolate was shown to be homogeneous by tlc in three different solvent systems, namely, solvents 1 (R_f 0.39), 2 (R_f 0.14), and 3, *n*-hexane–Et_2O–iPrOH–EtOH–H_2O (6:16:9:10:9) (upper layer, R_f 0.57). Fraction 12 (0.51 g) was repeatedly treated with a $CHCl_3$-MeOH (1:1) mixture, and the purified precipitate was collected by filtration to yield the amorphous isolate, abrusoside B [**2**] (150 mg, 0.027% dry wt), which was shown to be homogeneous by tlc in solvents 1–3 (R_f 0.64, 0.43, and 0.47, respectively). Fractions 28–29 were also repeatedly treated with $CHCl_3$-MeOH (1:1), and collection of the purified precipitate afforded abrusoside C [**3**] (220 mg, 0.040% dry wt), which was shown to be pure by tlc in solvents 1–3 (R_f 0.25, 0.06, and 0.50, respectively).

ISOLATION OF ABRUSOSIDE D [**4**].—The precipitate (15 g) obtained by adding MeOH to *n*-BuOH extract III was further fractionated by dccc using an equilibrated mixture of $CHCl_3$–MeOH–*n*-PrOH–H_2O (6:4:1:5), with the upper phase employed as mobile phase. The solute (1.5 g) was dissolved in 5.0 ml mobile phase and, on the addition of 5.0 ml of stationary phase, was introduced into a 10-ml sample chamber. Ascending development was employed as a pressure of 2–4 kg/cm^2. Fractions (300 drops each) were collected and monitored by tlc using solvents 1 and 2. This procedure was repeated once. Fractions 181–240 of each dccc run contained a compound shown to be pure by tlc in solvents 1 (R_f 0.09), 3 (R_f 0.30), and 4, $CHCl_3$-MeOH-EtOH-40% HOAc (8:3:3:2) (R_f 0.34) and were combined to yield abrusoside D [**4**] (320 mg, 0.289% dry wt).

CHARACTERIZATION OF ABRUSOSIDE A [**1**].—This isolate exhibited the following data: mp 278–280°; $[\alpha]_D$ +11.2° (c = 0.31, pyridine); uv (EtOH) end absorption; ir ν max (KBr) 3412 (OH), 1713 (C=O), 1127, 1076, 1045 cm^{-1}; ^{1}H nmr (360 MHz, C_5D_5N) δ 6.58 (1H, m, H-24), 5.14 (1H, d, J = 7.7 Hz, H-1′), 4.72 (1H, dd, J = 11.7 Hz, J = 4.3 Hz, H-3), 4.53 (2H, m, H-6′, H-22), 4.40 (1H, dd, J = 11.7 Hz, J = 4.9 Hz, H-6′), 4.27 (2H, m, H-3′, H-4′), 4.00 (2H, m, H-2′, H-5′), 1.95 (3H, s, Me-27), 1.61 (3H, s, Me-30), 1.01 (3H, d, J = 6.6 Hz, Me-21), 0.95 (3H, s, Me-18), 0.81 (3H, s, Me-28), 0.57, 0.28 (2H, d, J = 3.5 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, C_5D_5N) δ 180.19 (s, C-29), 166.34 (s, C-26), 140.71 (d, C-24), 127.65 (s, C-25), 105.50 (d, C-1′), 85.31 (d, C-3), 80.36 (d, C-22), 78.23 (d, C-5′), 77.95 (d, C-3′), 75.38 (d, C-2′), 71.40 (d, C-4′), 62.64 (t, C-6′), 54.39 (s, C-4), 48.98 (s, C-14), 48.02 (d, C-8), 47.83 (d, C-17), 45.30 (s, C-13), 44.70 (d, C-5), 40.04 (d, C-20), 35.50 (t, C-12), 32.93 (t, C-15), 31.79 (t, C-1), 29.73 (t, C-19), 29.29 (t, C-2), 27.87 (t, C-23), 27.49 (t, C-7), 26.44 (t, C-11), 25.84 (t, C-16), 25.51 (s, C-10), 23.10 (t, C-6), 19.78 (s, C-9), 19.46 (q, C-28), 18.00 (C-18), 17.28 (q, C-27), 13.11 (q, C-21), 11.08 (q, C-30) ppm; eims (70 eV) *m/z* [M]$^+$ of aglycone 484 (1%), 469 (1), 466 (1), 448 (1), 423 (1), 405 (1), 385 (1), 367 (1), 345 (5), 327 (9), 314 (6), 299 (40), 281 (3), 175 (18), 159 (21), 147 (24), 133 (31), 121 (41), 111 (30), 107 (48), 95 (100), 81 (40), 55 (54), 44 (89); fabms (DTE/DTT) *m/z* [M + Na]$^+$ 669; fabms (LiI/3-NBA) *m/z* [M + Li]$^+$ 653; hr fabms mass measurement found 669.3608, calcd for $C_{36}H_{54}O_{10}Na$, 669.3615.

Abrusoside A [**1**] (30 mg) was hydrolyzed by treatment with 1 N HCl (8 ml) for 4 h at 100° to produce an aglycone, abrusogenin [**5**] (21 mg), which was recrystallized from MeOH as colorless, needle-shaped crystals and shown to be homogeneous by tlc in solvents 2 (R_f 0.35), 5, cyclohexane-EtOAc-$CHCl_3$-MeOH (6:4:2:3) (R_f 0.49), and 6, $CHCl_3$-MeOH (10:1) (R_f 0.23). The H_2O layer containing the saccharide portion of abrusoside A [**1**] was neutralized with Ag_2CO_3 with the filtrate subjected to gc-ms and tlc, and found to contain only D-glucose. Abrusogenin [**5**] exhibited the following data: mp 278–280°; $[\alpha]_D$ +37° [c = 0.1, $CHCl_3$-MeOH, (1:1)]; uv (EtOH) end absorption; ir ν max (KBr) 3430 (OH), 1707 (C=O), 1384, 1259, 1147, 1046 cm^{-1}; ^{1}H nmr (360 MHz, $CDCl_3$ + CD_3OD) δ 6.63 (1H, m, H-24), 4.50 (1H, dd, J = 13.2 Hz, J = 2.8 Hz, H-22), 4.09 (1H, dd, J = 11.1 Hz, J = 4.3 Hz, H-3), 2.58 (1H, m, H-23$_a$), 1.91 (3H, br s, Me-27), 1.13 (3H, s, Me-30), 1.00 (3H, d, J = 6.7 Hz, Me-21), 0.97 (3H, s, Me-18), 0.93 (3H, s, Me-28), 0.61, 0.39 (2H, d, J = 4.0 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, $CDCl_3$)

δ 180.19 (s, C-29), 167.13 (s, C-26), 140.29 (d, C-24), 127.96 (s, C-25), 80.51 (d, C-22), 75.22 (d, C-3), 54.57 (s, C-4), 48.87 (s, C-14), 47.92 (d, C-8), 47.54 (d, C-17), 45.24 (s, C-13), 44.10 (d, C-5), 40.02 (d, C-20), 35.44 (t, C-12), 32.68 (t, C-15), 31.58 (t, C-1), 29.92 (t, C-19), 29.21 (t, C-2), 27.86 (t, C-23), 27.58 (t, C-7), 26.37 (t, C-11), 25.60 (t, C-16), 25.09 (s, C-10), 23.00 (t, C-6), 19.95 (s, C-9), 19.37 (q, C-28), 17.89 (q, C-18), 17.07 (q, C-27), 12.85 (q, C-21), 9.18 (q, C-30) ppm; eims (70 eV) *m/z* $[M]^+$ 484 (10%), $[M-Me]^+$ 469 (16), $[M-H_2O]^+$ 466 (19), 448 (17), 423 (11), 385 (19), 367 (11), 344 (10), 314 (81), 299 (18), 233 (28), 215 (22), 203 (15), 187 (28), 173 (38), 161 (44), 147 (52), 119 (59), 111 (58), 107 (81), 95 (100), 93 (67), 91 (38), 81 (50), 79 (32), 69 (24), 67 (25), 55 (80); hrms mass measurement found 484.3189 (calcd for $C_{30}H_{44}O_5$, 484.3186), 367.2630 ($C_{25}H_{35}O_2$, 367.2635), 314.2245 ($C_{21}H_{30}O_2$, 314.2244), 181.1223 ($C_{11}H_{17}O_2$, 181.1227), 167.1072 ($C_{10}H_{15}O_2$, 167.1071), 139.0764 ($C_8H_{11}O_2$, 139.0768), 111.0445 ($C_6H_7O_2$, 111.0445), 95.0131 ($C_5H_3O_2$, 95.0133).

Abrusogenin [**5**] (10 mg) was methylated in ethereal CH_2N_2 at room temperature overnight. On workup, abrusogenin methyl ester [**6**] (8 mg) was obtained as colorless prisms after recrystallization in EtOAc and was shown to be pure by tlc in solvents 2 (R_f 0.71), 5 (R_f 0.56), and 6 (R_f 0.60). This derivative exhibited the following data: mp 246–248°; $[\alpha]_D$ +31.2° [*c* = 0.08, $CHCl_3$-MeOH (1:1)]; uv (EtOH) end absorption; ir ν max (KBr) 3529 (OH), 1710 (C=O), 1258, 1135, 1060, 1018 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 6.61 (1H, m, H-24), 4.50 (1H, dd, *J* = 13.4 Hz, *J* = 2.8 Hz, H-22), 4.10 (1H, dd, *J* = 11.3 Hz, *J* = 4.4 Hz, H-3), 3.71 (3H, s, 29-OMe), 2.57 (1H, m, H-23_a), 1.91 (3H, br s, Me-27), 1.15 (3H, s, Me-30), 1.00 (3H, d, *J* = 6.8 Hz, Me-21), 0.96 (3H, s, Me-18), 0.93 (3H, s, Me-28), 0.61, 0.39 (2H, d, *J* = 4.2 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 177.42 (s, C-29), 166.56 (s, C-26), 139.65 (d, C-24), 128.12 (s, C-25), 80.21 (d, C-22), 75.32 (d, C-3), 54.80 (s, C-4), 51.85 (q, Me-29), 48.79 (s, C-14), 47.85 (d, C-8), 47.42 (d, C-17), 45.20 (s, C-13), 44.30 (d, C-5), 40.05 (d, C-20), 35.40 (t, C-12), 32.58 (t, C-15), 31.43 (t, C-1), 29.88 (t, C-19, C-2), 27.85 (t, C-23), 27.55 (t, C-7), 26.32 (t, C-11), 25.52 (t, C-16), 25.04 (s, C-10), 23.07 (t, C-6), 19.91 (s, C-9), 19.38 (q, C-28), 17.86 (q, C-18), 17.14 (q, C-27), 12.82 (q, C-21), 9.26 (q, C-30) ppm; eims (70 eV) *m/z* $[M]^+$ 498 (1%), $[M-OH]^+$ 480 (3), 465 (1), 448 (4), 421 (1), 367 (4), 341 (3), 314 (22), 299 (6), 247 (11), 215 (14), 199 (11), 173 (30), 147 (42), 133 (55), 119 (60), 111 (68), 107 (76), 105 (68), 95 (100), 81 (72), 67 (39), 55 (48).

Abrusogenin [**5**] (10 mg) was acetylated overnight at room temperature in pyridine-Ac_2O (1:1) (1.0 ml). On workup, the product abrusogenin 3-monoacetate [**6**] (8 mg) was recrystallized as colorless needles from MeOH. This compound was shown to be homogeneous by tlc in three solvent systems (solvents 2, 5, and 6; R_f 0.64, 0.43, and 0.47, respectively) and exhibited the following data: mp 308–310°; $[\alpha]_D$ +37.4° [*c* = 0.06, $CHCl_3$-MeOH (1:1)]; uv (EtOH) end absorption; ir ν max (KBr) 1724 (C=O), 1703 (C=O), 1383, 1285, 1250, 1137 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$ + CD_3OD) δ 6.66 (1H, m, H-24), 5.25 (1H, dd, *J* = 11.5 Hz, *J* = 4.3 Hz, H-3), 4.51 (1H, dd, *J* = 12.6 Hz, *J* = 2.4 Hz, H-22), 2.58 (1H, m, H-23_a), 2.01 (3H, s, -OAc), 1.91 (3H, br s, Me-27), 1.20 (3H, s, Me-30), 1.00 (3H, d, *J* = 6.7 Hz, Me-21), 0.98 (3H, s, Me-18), 0.94 (3H, s, Me-28), 0.64, 0.42 (2H, d, *J* = 4.1 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, $CDCl_3$ + CD_3OD) δ 178.51 (s, C-29), 170.79 (s, -OAc), 167.13 (s, C-26), 140.26 (d, C-24), 128.01 (s, C-25), 80.53 (d, C-22), 77.84 (d, C-3), 52.74 (s, C-4), 48.85 (s, C-14), 47.91 (d, C-8), 47.56 (d, C-17), 45.28 (s, C-13), 44.31 (d, C-5), 40.04 (d, C-20), 35.45 (t, C-12), 32.64 (t, C-15), 31.22 (t, C-1), 29.89 (t, C-19), 27.85 (t, C-23), 27.59 (t, C-7), 26.37 (t, C-11), 26.12 (t, C-2), 25.49 (t, C-16), 25.09 (s, C-10), 22.78 (t, C-6), 21.19 (q, -OAc), 20.17 (s, C-9), 19.36 (q, C-28), 17.91 (q, C-18), 17.07 (q, C-27), 12.98 (q, C-21), 10.34 (q, C-30) ppm; eims (70 eV) *m/z* $[M]^+$ 526 (3%), 511 (3), $[M-OAc]^+$ 466 (16), 448 (15), 427 (7), 405 (6), 367 (11), 314 (19), 299 (5), 233 (17), 215 (11), 187 (13), 173 (19), 159 (25), 147 (28), 121 (29), 119 (34), 111 (33), 107 (43), 105 (37), 133 (34), 95 (71), 81 (38), 67 (26), 55 (69), 43 (100).

CHARACTERIZATION OF ABRUSOSIDE B [**2**].—Abrusoside B [**2**] exhibited the following data: mp 243–245°; $[\alpha]_D$ +5.8° (*c* = 0.35, pyridine); uv (EtOH) end absorption; ir ν max (KBr) 3407 (OH), 1712 (C=O), 1378, 1245, 1114, 1081, 1059 cm^{-1}; ^1H nmr (360 MHz, C_5D_5N) δ 6.56 (1H, m, H-24), 5.37 (1H, d, *J* = 7.6 Hz, H-1″), 5.28 (1H, br d, W½ = 7.1 Hz, H-1′), 4.87 (dd, *J* = 11.7 Hz, *J* = 4.4 Hz, H-3), 3.86 (3H, s, OMe-6′), 1.94 (3H, br s, Me-27), 1.71 (3H, s, Me-30), 1.01 (3H, d, *J* = 6.5 Hz, Me-21), 0.96 (3H, s, Me-18), 0.81 (3H, s, Me-28), 0.60, 0.30 (2H, d, *J* = 3.5 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, C_5D_5N) δ 179.52 (s, C-29), 170.35 (s, C-6′), 166.21 (s, C-26), 140.51 (d, C-24), 127.71 (s, C-25), 106.35 (d, C-1″), 102.47 (d, C-1′), 84.05 (d, C-2′), 82.59 (d, C-3), 80.28 (d, C-22), 78.15 (d, C-5″), 78.05 (d, C-5′), 77.70 (d, C-3′), 77.43 (d, C-3″), 76.15 (d, C-2″), 72.87 (d, C-4′), 71.32 (d, C-4″), 62.59 (t, C-6″), 54.16 (s, C-4), 51.99 (q, OMe-6′), 48.93 (s, C-14), 48.03 (d, C-8, C-17), 45.32 (s, C-13), 45.19 (d, C-5), 40.07 (d, C-20), 35.58 (d, C-12), 32.96 (t, C-15), 31.88 (t, C-1), 29.73 (t, C-19), 29.63 (t, C-2), 27.88 (t, C-23), 27.52 (t, C-7), 26.41 (t, C-11), 25.97 (t, C-16), 25.39 (s, C-10), 23.21 (t, C-6), 19.77 (s, C-9), 19.48 (q, C-28), 18.10 (q, C-18), 17.28 (q, C-27), 13.11 (q, C-21), 10.60 (q, C-30) ppm; eims (70 eV) *m/z* $[M]^+$ of aglycone 484 (3%), 469 (3), 466 (4), 448 (5), 438 (3), 423 (3), 405 (2),

385 (4), 367 (4), 314 (14), 299 (4), 233 (6), 173 (14), 147 (13), 121 (18), 95 (33), 73 (20), 55 (38), 44 (100); fabms, (DTE/DTT) *m/z* 859 $[M+Na]^+$; fabms, (3-NBA) *m/z* $[M+H]^+$ 837, $[M+Na]^+$ 859; fabms (LiI/3-NBA) *m/z* $[M+Li]^+$ 843, $[M-H+2\ Li]^+$ 849; hr fabms mass measurement found 837.4271, calcd for $C_{43}H_{65}O_{16}$, 837.4273.

Abrusoside B [**2**] (5 mg) was hydrolyzed with 1 N HCl (3 ml) under the same conditions described for abrusoside A [**1**]. On workup, the resultant aglycone (2.8 mg) was shown to be identical to abrusogenin [**5**] by physical (mmp), spectral (ir ^{1}H nmr, ms), and tlc data comparison. On neutralization of the H_2O layer obtained from this hydrolysis, the sugars identified by gc-ms and tlc were D-glucose and D-glucuronic acid methyl ester. The latter compound was generated from authentic D-glucuronic acid by methylation using CH_2N_2.

CHARACTERIZATION OF ABRUSOSIDE C [**3**].—Compound **3** exhibited the following data: mp 260–262°; $[\alpha]_D$ +31.4° (*c* = 0.34, pyridine); uv (EtOH) end absorption; ir ν max (KBr) 3412 (OH), 1709 (C=O), 1379, 1259, 1077 cm^{-1}; ^{1}H nmr (360 MHz, C_5D_5N) δ 6.56 (1H, m, H-24), 5.23 (1H, d, *J* = 7.7 Hz, H-1″), 5.15 (1H, d, *J* = 7.6 Hz, H-1′), 4.70 (1H, dd, *J* = 11.7 Hz, *J* = 4.3 Hz, H-3), 1.94 (3H, br s, Me-27), 1.66 (3H, s, Me-30), 1.01 (3H, d, *J* = 6.6 Hz, Me-21), 0.94 (3H, s, Me-18), 0.79 (3H, s, Me-28), 0.56, 0.28 (2H, d, *J* = 3.4 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, C_5D_5N) δ 178.86 (d, C-29), 165.83 (s, C-26), 139.97 (d, C-24), 128.10 (s, C-25), 105.80 (d, C-1″), 103.63 (d, C-1′), 84.96 (d, C-3), 83.88 (d, C-2′), 80.45 (d, C-22), 78.34 (d, C-3″), 78.03 (d, C-5′, C-5″), 77.59 (d, C-3′), 76.60 (d, C-2″), 71.84 (d, C-4″), 71.25 (d, C-4′), 63.16 (t, C-6″), 62.62 (t, C-6′), 54.49 (s, C-4), 49.17 (s, C-14), 48.36 (d, C-8), 47.68 (d, C-17), 45.71 (s, C-13), 44.92 (d, C-5), 40.34 (d, C-20), 35.67 (t, C-12), 33.27 (t, C-15), 31.98 (t, C-1), 29.63 (t, C-19), 29.56 (t, C-2), 28.09 (t, C-23), 27.62 (t, C-7), 26.79 (t, C-11), 25.87 (s, C-16), 25.79 (t, C-10), 23.02 (t, C-6), 20.21 (s, C-9), 19.56 (q, C-28), 17.90 (q, C-18), 17.00 (q, C-27), 13.23 (q, C-21), 10.87 (q, C-30) ppm; eims (70 eV) *m/z* $[M]^+$ of aglycone 484 (2%), 469 (4), 466 (4), 448 (4), 423 (3), 405 (3), 385 (5), 367 (3), 314 (18), 299 (5), 255 (1), 233 (6), 173 (24), 145 (23), 133 (22), 119 (21), 111 (23), 107 (31), 105 (23), 95 (62), 73 (76), 54 (72), 43 (100); fabms (DTE/DTT) *m/z* $[M+Na]^+$ 831; fabms (LiI/3-NBA) *m/z* $[M+Li]^+$ 815, $[M-H+2\ Li]^+$ 821; hr fabms mass measurement found 815.4406, calcd for $C_{42}H_{64}O_{15}Li$, 815.4408.

Abrusoside C [**3**] (5 mg) was acid-hydrolyzed and worked up by the method described for abrusoside A [**1**]. The aglycone obtained was identical to abrusogenin by mmp, ir, ^{1}H nmr, ms, and co-tlc, and only D-glucose was detected by gc-ms and tlc.

CHARACTERIZATION OF ABRUSOSIDE D [**4**].—Compound **4** exhibited the following data: mp 237–239°; $[\alpha]_D$ +9.9° (*c* = 0.31, pyridine); uv (EtOH) end absorption; ir ν max (KBr) 3412 (OH), 1710 (C=O), 1379, 1258, 1115, 1077, 1054 cm^{-1}; ^{1}H nmr (360 MHz, C_5D_5N) δ 6.56 (1H, m, H-24), 5.44 (1H, d, *J* = 7.4 Hz, H-1′), 5.22 (1H, d, *J* = 7.2 Hz, H-1″), 4.83 (1H, dd, *J* = 11.7 Hz, *J* = 4.0 Hz, H-3), 4.74 (1H, d, *J* = 10 Hz, H-5′), 1.94 (3H, br s, Me-27), 1.70 (3H, s, Me-30), 1.01 (3H, d, *J* = 6.6 Hz, Me-21), 0.92 (3H, s, Me-18), 0.78 (3H, s, Me-28), 0.50, 0.23 (2H, d, *J* = 3.5 Hz, H_2-19) ppm; ^{13}C nmr (90.8 MHz, C_5D_5N) δ 179.54 (s, C-29), 173.69 (s, C-6′), 166.21 (s, C-26), 140.58 (d, C-24), 127.68 (s, C-25), 105.77 (d, C-1″), 102.59 (d, C-1′), 83.71 (d, C-3), 83.08 (d, C-2′), 80.29 (d, C-22), 78.07 (d, C-3″, C-5″), 77.70 (d, C-5′), 77.30 (d, C-3′), 76.52 (d, C-2″), 73.19 (d, C-4′), 71.29 (d, C-4″), 62.56 (t, C-6″), 54.18 (s, C-4), 48.91 (s, C-14), 48.03 (d, C-8), 47.92 (d, C-17), 45.30 (s, C-13), 44.86 (d, C-5), 40.06 (d, C-20), 35.52 (t, C-12), 32.39 (t, C-15), 31.79 (t, C-1), 29.71 (t, C-19), 29.23 (t, C-2), 27.89 (t, C-23), 27.50 (t, C-7), 26.39 (t, C-11), 25.86 (t, C-16), 25.44 (t, C-10), 23.12 (t, C-6), 19.78 (t, C-9), 19.47 (q, C-28), 18.04 (q, C-18), 17.29 (q, C-27), 13.12 (q, C-21), 11.00 (q, C-30) ppm; eims (70 eV) *m/z* [M of aglycone − Me]$^+$ 469 (1%), 448 (2), 423 (1), 405 (2), 385 (1), 367 (2), 314 (8), 299 (2), 233 (5), 173 (15), 145 (17), 119 (20), 95 (36), 73 (34), 44 (100); fabms (DTT/DTE) *m/z* $[M+H]^+$ 823, $[M+Na]^+$ 845; hr fabms mass measurement found 823.4123, calcd for $C_{42}H_{63}O_{16}$, 823.4116.

When abrusoside D [**4**] (7 mg) was acid-hydrolyzed as described for abrusoside A [**1**], abrusogenin [**5**] (2.9 mg) (mmp, ir, ^{1}H nmr, ms, co-tlc), D-glucose, and D-glucuronic acid (gc-ms, tlc) were the products identified.

PRELIMINARY SAFETY EVALUATION OF *A. PRECATORIUS* MEOH EXTRACT AND ABRUSOSIDES A–D.—The initial MeOH/H_2O extract from *A. precatorius* leaves was tested for acute toxicity in male Swiss-Webster mice, administered by oral intubation at dose levels of 1 and 2 g/kg body wt. The pure isolates, abrusosides A–D, were tested in this manner at only a single dose of 1.0 g/kg, due to the limited amount of each isolate available. The procedures and protocols for toxicological testing were followed as published previously (43–45). None of the samples tested caused any lethality, and body weights recorded on days 0 (prior to administration), 1, 3, 7, and 14 did not differ significantly for treated versus control animals.

The mutagenic potentials of the *A. precatorius* leaf initial MeOH/H_2O extract and of pure abrusosides A–D were evaluated in the dose ranges 5.0–10.0 and 2.5–5.0 mg/ml, respectively, according to estab-

lished protocols (43–46). None of these test materials was mutagenic for *S. typhimurium* strain TM677, either in the presence or absence of a metabolic activating system derived from the livers of Aroclor 1254-pretreated rats.

SENSORY EVALUATION OF *A. PRECATORIUS* MEOH EXTRACT AND ABRUSOSIDES A–D.—A small portion (< 0.1 mg) of the initial MeOH/H_2O extract of *A. precatorius* leaves was briefly tasted and then expectorated, and thereby established as being slightly sweet. Abrusosides A–D were evaluated in the form of their H_2O-soluble ammonium salts by a small taste panel consisting of three persons (44,45). Each compound was dissolved in aqueous NH_4OH solution (ca. 0.05 N), and dilutions in H_2O were compared in sweetness intensity to a standard 2% w/v aqueous solution of sucrose. Abrusosides A [**1**], B [**2**], C [**3**], and D [**4**] were rated as possessing about 30, 100, 50, and 75 times the sweetness intensity of 2% w/v sucrose, respectively. All of the compounds, however, exhibited a lingering aftertaste. Compounds **5** through **7** were devoid of sweetness, and were not tasted by the test panel.

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