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ABRUSOSIDE E, A FURTHER SWEET-TASTING CYCLOARTANE GLYCOSIDE FROM THE LEAVES OF *ABRUS PRECATORIUS**

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Key Word Index—*Abrus precatorius*; Fabaceae; cycloartane glycoside; abrusoside E; sweet taste.

Abstract—A novel cycloartane glycoside, designated abrusoside E, has been isolated from the leaves of *Abrus precatorius*. Its structure was determined as 3β-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-glucopyranosyl](20*S*,22*S*)-3β,22-dihydroxy-9,19-cyclolanost-24-en-26,29-dioic acid δ-lactone based upon spectroscopic methods carried out on the parent compound and its mono- and dimethyl esters. While abrusoside E was found to be only marginally sweet, the monomethyl ester proved to be more potently sweet-tasting.

INTRODUCTION

Abrus precatorius L. is a weedy subtropical vine with leaves that are known to be sweet-tasting [2]. Although the sweetness was once attributed to the presence of the well-known sweet-tasting oleanane glycoside, glycyrrhizin, recent work in this laboratory resulted in the isolation from the leaves of four potently sweet cycloartane glycosides, designated abrusosides A–D, while glycyrrhizin was not detected [3, 4]. These four compounds share a common aglycone, namely, abrusogenin, whose structure was confirmed by X-ray crystallography, and differ in the saccharide moieties attached to the C-3 position; they have been rated as being 30–100 times more potently sweet than sucrose on a weight basis [3, 4]. These sweet compounds are not acutely toxic for mice, or mutagenic for bacteria, and may be rendered water-soluble by conversion to their ammonium salts [3, 4]. Abrusosides A–D were also found to occur in the leaves of *A. fruticulosus* collected in Thailand [5].

As part of a continuing effort to identify additional sweet compounds of this type, we report herein the isolation and characterization of a further novel cycloartane glycoside, abrusoside E (**1**), from the leaves of *A. precatorius* collected in Florida, U.S.A. For structural elucidation purposes, it was necessary to prepare the mono- (**2**) and dimethyl esters (**3**) of **1** in this investigation.

RESULTS AND DISCUSSION

A defatted methanol extract of the leaves of *A. precatorius* was dried under reduced pressure, and partitioned between *n*-butanol and water. The *n*-butanol portion (106 g) was then chromatographed over silica gel with a gradient of chloroform, methanol and water. A precipitate was observed in fractions eluted with chloroform–methanol–water (65:38:10), and these fractions were filtered. The TLC characteristics (R_f and chromogenic response to vanillin-H_2SO_4) of the precipitate were very similar to a standard sample of abrusoside D. Repeated silica gel chromatography of this material resulted in a sparingly soluble pure compound, abrusoside E (**1**). The negative FAB-mass spectrum of **1** exhibited a pseudomolecular ion $[M - H]^{-1}$ at *m/z* 821, with the molecular formula of $C_{42}H_{62}O_{16}$ being established by HRFAB-mass spectrometry. This molecular formula is the same as that of abrusoside D. However, the fragment ions at *m/z* 645 $[M - H - 176]^-$ and 483 $[M - H - 176 - 162]^-$ were indicative of the sequential losses of glucuronic acid and glucose [6], thus suggesting that compound **1** contained a 3-*O*-β-D-glucuronopyranosyl-(1 → 2)-β-D-glucopyranosyl subunit, rather than the reverse glycosidic order as found in abrusoside D [4]. Acid hydrolysis of compound **1** resulted in an aglycone that was identified by TLC, $[\alpha]_D$, mp, ^{1}HNMR, and ^{13}CNMR as the known compound, abrusogenin. Also, GC–mass spectral analysis of the resulting free sugars led to the detection of only two sugars, D-glucose and D-glucuronic acid, in a 1:1 ratio.

Partial methylation of **1** was accomplished by refluxing with methanol and 1N HCl to yield the monomethyl ester, **2**. Unlike the parent compound, this derivative proved to be soluble in pyridine-d_5, and the resulting spectroscopic data were similar to those of abrusoside D [4], with **2** containing one additional carbon at δ51.9 (*q*), indicating the presence of a methyl ester. The FAB-mass spectrum of **2** displayed a pseudomolecular ion at *m/z* 835, confirming the presence of the methyl ester,

* Part 31 in the series 'Potential Sweetening Agents of Plant Origin'. For Part 30, see ref. [1].

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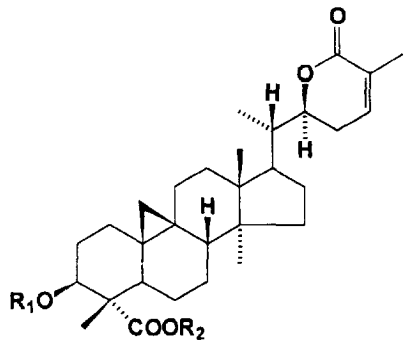

	R_1	R_2
1	β-D-glc-β-D-glcA	H
2	β-D-glc-β-D-glcA-6-CH_3	H
3	β-D-glc-β-D-glcA-6-CH_3	CH_3

while fragments at *m/z* 645 $[M - H - GlcA\text{-}6\text{-}CH_3]^-$ and 483 $[M - H - Glc\text{-}6\text{-}CH_3 - Glc]^-$ suggested the presence of a 3-*O*-β-D-methylglucuronopyranosyl-(1 → 2)-β-D-glucopyranosyl saccharide moiety.

The assignments of the ^1H and ^{13}C NMR spectra of **2** were facilitated by direct correlation NMR experiments including ^1H–^1H COSY and ^1H–^{13}C HETCOR. The selective INEPT experiment [7] was used to confirm the position of the methyl ester substituent in **2**, by irradiation of the OCH_3 signal ($^3J_{CH} = 4$ Hz), wherein C-6″ (δ170.3) was selectively enhanced. The assignments of the two anomeric protons were unambiguously determined with the help of the ^1H–^1H HOHAHA NMR experiment [8] in which one of the methylene protons of glucose (1H, δ4.4, $J = 5$ and 12 Hz, H-6′) clearly correlated to the anomeric proton at δ5.37 (1H, *d*, $W_{1/2} = 7.2$ Hz, H-1′). In addition to the above-mentioned two-dimensional NMR experiments, comparison with published data for model glycosides [4, 9] was used to confirm the ^{13}C NMR assignments of **2**. The anomeric configurations of the glucose and glucuronic acid units were both determined to be β according to the *J* values of their proton signals (7.2 and 7.5 Hz, respectively). A ^1H–^1H ROESY NMR experiment displayed a NOE between the anomeric proton of glucose at δ5.37 and H-3 of the genin at δ4.96, also indicating that the glucopyranosyl unit was directly attached to the genin. Further confirmation of the composition of the saccharide subunit was obtained through selective INEPT experiments. Irradiation of H-3 (δ4.96, $^3J_{CH} = 6$ Hz) resulted in the enhancement of C-1′ (δ102.5) and also C-29 (δ179.5) and C-30 (δ10.6), and irradiation of H-1″ (δ5.45, $^3J_{CH} = 7.5$ Hz) selectively enhanced C-2′ (δ84.1), thus indicating a 1″ → 2′ intersaccharide linkage. This saccharide linkage was confirmed by comparing published data for model glycosides [4]. Finally, a small amount of **2** was methylated using ethereal diazomethane, which resulted in two methoxyl group signals appearing in the ^{13}C NMR spectrum (δ51.9 and 51.8) of **3**, thus confirming the presence of the carboxylic acid functional group on the cycloartane skeleton.

Compound **1** represents the fifth naturally occurring sweet-tasting compound based on the aglycone abrusogenin isolated from *Abrus precatorius*. Abrusoside E (**1**) was only marginally sweet in contrast to its isomer, abrusoside D [4], but the monomethyl ester, **2**, exhibited increased sweetness intensity in addition to improved hedonic qualities when compared with **1**. The dimethyl ester (**3**) of abrusoside E (**1**) had no sweet taste.

EXPERIMENTAL

Mps: uncorr.; $[\alpha]_D$: at 25° (Perkin-Elmer Model 241). IR spectra: thin films (Midac Collegian FT-IR spectrometer). UV spectra: MeOH soln (Beckman DU-7 spectrometer). NMR spectra: Varian XL-300, Nicolet NT-360 and GE Omega 500 MHz NMR spectrometers, TMS as int. standard. FAB-MS and HRFAB-MS: Finnigan MAT-90 instrument, glycerol matrix.

Plant material. The leaves of *A. precatorius* were collected in Miami, Florida, U.S.A. in June, 1993.

Extraction and isolation. The air-dried leaves (1.5 kg) were extracted with MeOH, defatted with petrol, and the methanolic soln evapd under red. pres., and partitioned between *n*-BuOH and H_2O. The resulting *n*-BuOH extract was dried under red. pres., yielding 107 g. A portion (106 g) of the *n*-BuOH extract was sepd over silica gel by CC using $CHCl_3$ and mixtures of $CHCl_3$–MeOH and $CHCl_3$–MeOH–H_2O as eluants. Several adjacent fractions of the $CHCl_3$–MeOH–H_2O (65:38:10) elution contained a ppt. which was filtered. This ppt. was further purified by repeated silica gel flash chromatography with $CHCl_3$–MeOH (3:2) and $CHCl_3$–MeOH–H_2O (6:4:1) as mobile phases, yielding a single compound, abrusoside E (**1**).

Abrusoside E (3β-O-[β-D-*glucuronopyranosyl*-(1 → 2)-β-D-*glucopyranosyl*] (20S,22S)-3β,22-*dihydroxy*-9,19-*cyclolanost*-24-*en*-26,29-*dioic acid* δ-*lactone*) (**1**). White, amorphous powder, mp 265° (dec.). $[\alpha]_D$ + 2° (pyridine, *c* 0.2). IR ν_{max} cm^{-1}: 3402 (OH), 1693 (C=O), 1601, 1535, 1393. UV λ_{max}^{MeOH} end absorption. FAB-MS (-ve) *m/z*: 821 $[M - H]^-$, 645 $[M - 176]^-$, 483 $[M - 176 - 162]^-$. HRFAB-MS *m/z* found 823.41340 calcd for $C_{42}H_{63}O_{16}$, 823.41161.

Acid hydrolysis of compound **1**. Abrusoside E (**1**) was hydrolysed by refluxing with 1N HCl for 2 hr. Excess H_2O was then added to the reaction mixture, and a ppt. formed, which was filtered then chromatographed over silica gel using 6% MeOH in $CHCl_3$ as the solvent system to yield the known aglycone, abrusogenin. Short needles; mp 304–306° (dec.) (ref. [3, 4] mp 278–280°). $[\alpha]_D$ + 21° (pyridine, *c* 0.2) (ref. [3, 4] + 37°) $[\alpha]_D$ (pyridine, *c* 0.1). IR, UV, ^1H, ^{13}C NMR, MS in agreement with published data [3, 4].

The aq. extract was evapd *in vacuo*, and the residue was then treated with Sigma-Sil-A (Sigma Chemical Co., St. Louis) for 1 hr at 65°. The resulting silylated sample was analysed by GC–MS with the initial column temperature of 120°, increased by 8° min^{-1} to 270°. The injector and interface temperatures were maintained at 220° and 280°, respectively. The mass spectrometer was in the EI mode, with a voltage of 70 eV, a current of 0.25 mA, and a set time of 1 sec. Standard samples of TMSi–D-glucose and TMSi–D-glucuronic acid were prepared in the same manner as the aq. extract.

Partial methylation of **1**. 3β-*O*-[β-D-Methylglucuronopyranosyl-(1 → 2)-β-D-glucopyranosyl] (20*S*,22*S*)-3β,22-dihydroxy-9,19-cycloanost-24-en-26,29-dioic acid δ-lactone (**2**). Compound **1** (100 mg) was dissolved in 5 ml of MeOH, then 5 ml of 1N HCl were added, and the reaction was refluxed overnight to provide **2**, a monomethyl ester of abrusoside E. White amorphous powder, mp 258° (dec.). $[\alpha]_D$ − 2° (pyridine, *c* 0.2). IR ν_{max} cm^{-1}: 3373 (OH), 1716 (C=0), 1456, 1385, 1259, 1049. UV λ_{max}^{MeOH}: end absorption. ^{1}H NMR (500 MHz, C_5D_5N): δ6.59 (1H, *m*, H-24), 5.45 (1H, *d*, *J* = 7.5 Hz, H-1″), 5.37 (1H, *d*, $W_{1/2}$ = 7.2 Hz, H-1′), 4.96 (1H, *dd*, *J* = 4 and 12 Hz, H-3), 4.61 (1H, *d*, *J* = 9.5 Hz, H-5″), 4.60–4.53 (3H, overlapping signals, H-4″, 6′$_a$, and 22), 4.40 (1H, *dd*, *J* = 5 and 12 Hz, H-6′$_b$), 4.29 (1H, *t*, *J* = 9 Hz, H-3″), 4.24–4.19 (4H, overlapping signals, H-2′, 3′, 4′, and 2″), 3.92 (3H, *s*, OMe), 2.52 (1H, *bt*, *J* = 15.5 Hz, H-23) 2.01 (3H, *s*, Me-27), 1.79 (3H, *s*, Me-30) 1.07 (3H, *d*, *J* = 7 Hz, Me-21), 1.02 (3H, *s*, Me-18), 0.87 (3H, *s*, Me-28), 0.67 (1H, *d*, *J* = 3.8 Hz, H-19a), 0.37 (1H, *d*, *J* = 3.8 Hz, H-19b). ^{13}C NMR (90 MHz, C_5D_5N): δ179.5 (*s*, C-29), 170.3 (*s*, C-6″), 166.1 (*s*, C-26), 140.4 (*d*, C-24), 127.8 (*s*, C-25), 106.4 (*d*, C-1″), 102.5 (*d*, C-1′), 84.1 (*d*, C-2′), 82.6 (*d*, C-3), 80.2 (*d*, C-22), 78.2 (*d*, C-3′), 78.0 (*d*, C-5′), 77.7 (*d*, C-5″), 77.4 (*d*, C-3″), 76.2 (*d*, C-2″), 72.9 (*d*, C-4″), 71.3 (*d*, C-4′), 62.6 (*t*, C-6′), 54.2 (*s*, C-4), 51.9 (*q*, OMe), 48.9 (*s*, C-14), 48.0 (*d*, C-8), 48.0 (*d*, C-17), 45.3 (*s*, C-13), 45.2 (*d*, C-5), 40.1 (*d*, C-20), 35.6 (*t*, C-12), 33.0 (*t*, C-15), 31.9 (*t*, C-1), 29.6 (*t*, C-19), 29.5 (*t*, C-2), 27.9 (*t*, C-23), 27.5 (*t*, C-7), 26.4 (*t*, C-11), 26.0 (*t*, C-16), 25.4 (*s*, C-10), 23.2 (*t*, C-6), 19.8 (*s*, C-9), 19.5 (*q*, C-28), 18.1 (*q*, C-18), 17.3 (*q*, C-27), 13.1 (*q*, C-21), 10.6 (*q*, C-30). FAB-MS (-ve) *m/z*: 835 $[M - H]^-$, 645 $[M - 190]^-$, 483 $[M - 190 - 162]^-$.

Complete methylation of **1**. 3β-*O*-[β-D-Methylglucuronopyranosyl-(1 → 2)-β-D-glucopyranosyl] (20*S*,22*S*)-3β,22-dihydroxy-9,19-cycloanost-24-en-26,29-dioic acid δ-lactone (**3**). About 10 mg of **2** were methylated using ethereal CH_2N_2, and the resulting product (**3**) was purified over silica gel using 20% MeOH in $CHCl_3$ as the mobile phase. Amorphous white powder. UV λ_{max}^{MeOH}: end absorption. ^{1}H NMR (300 MHz, C_5D_5N): δ6.48 (1H, *m*, H-24), 5.25 (1H, *d*, *J* = 7.5 Hz, H-1″), 5.01 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.82 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 1.89 (3H, *s*, Me-27), 1.50 (3H, *s*, Me-30), 0.96 (3H, *d*, *J* = 7 Hz, Me-21), 0.90 (3H, *s*, Me-18), 0.78 (3H, *s*, Me-28), 0.50 (1H, *d*, *J* = 3.6 Hz, H-19a), 0.35 (1H, *d*, *J* = 3.5 Hz, H-19b). ^{13}C NMR (75 MHz, C_5D_5N): δ177.5 (*s*, C-29), 170.1 (*s*, C-6″), 166.0 (*s*, C-26), 140.3 (*d*, C-24), 127.8 (*s*, C-25), 106.6 (*d*, C-1″), 103.6 (*d*, C-1′), 84.3 (*d*, C-2′), 84.2 (*d*, C-3), 80.2 (*d*, C-22), 78.3 (*d*, C-3′), 78.0 (*d*, C-5′), 77.7 (*d*, C-5″), 77.5 (*d*, C-3″), 76.2 (*d*, C-2″), 72.8 (*d*, C-4″), 71.3 (*d*, C-4′), 62.7 (*t*, C-6′), 54.5 (*s*, C-4), 51.9 (*q*, OMe), 51.8 (*q*, OMe), 48.9 (*s*, C-14), 48.0 (*d*, C-8), 48.0 (*d*, C-17), 45.4 (*s*, C-13), 45.3 (*d*, C-5), 40.1 (*d*, C-20), 35.6 (*t*, C-12), 32.9 (*t*, C-15), 31.7 (*t*, C-1), 29.7 (*t*, C-19), 29.3 (*t*, C-2), 27.9 (*t*, C-23), 27.5 (*t*, C-7), 26.4 (*t*, C-11), 25.8 (*t*, C-16), 25.2 (*s*, C-10), 23.1 (*t*, C-6), 19.8 (*s*, C-9), 19.5 (*q*, C-28), 18.0 (*q*, C-18), 17.3 (*q*, C-27), 13.1 (*q*, C-21), 10.2 (*q*, C-30).

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REFERENCES

1. Kennelly, E. J., Cai, L., Long, L., Shamon, L., Zaw, K., Zhou, B.-N., Pezzuto, J. M. and Kinghorn, A. D. (1995) *J. Agric. Food Chem.*, **43**, 2602.
2. Inglett, G. E. and May, J. F. (1968) *Econ. Botany* **22**, 326.
3. Choi, Y.-H., Kinghorn, A. D., Shi, X., Zhang, H. and Teo, B. K. (1989) *J. Chem. Soc., Chem. Commun.*, 887.
4. Choi, Y.-H., Hussain, R. A., Pezzuto, J. M., Kinghorn, A. D. and Morton, J. F. (1989) *J. Nat. Prod.* **52**, 1118.
5. Fullas, F., Choi, Y.-H., Kinghorn, A. D. and Bunyapraphatsara, N. (1990) *Planta Med.* **56**, 332.
6. Wolfender, J.-L., Maillard, M., Marston, A. and Hostettmann (1992) *Phytochem. Anal.* **3**, 193.
7. Bax, A. (1984) *J. Magn. Reson.* **57**, 314.
8. Bax, A. and Davis, D. (1985) *J. Magn. Reson.* **65**, 355.
9. Kitagawa, I., Zhou, J. L., Sakagami, M., Taniyama, T. and Yoshikawa, M. (1988) *Chem. Pharm. Bull.* **36**, 3710.