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# Glycosyl Nervogenic Acid Esters of Carbohydrates from *Anodendron affine* (Anodendron. VI)[1)]

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Esters of *p*-*O*-glucosyl- and *p*-*O*-primverosylnervogenic acid with some carbohydrates (including dambonitol, glucose, and sucrose) were isolated from the seeds, unripened fruits, and caules of *Anodendron affine* DRUCE. The locations of the nervogenic acid moiety on dambonitol and sucrose were determined on the basis of spectral and chemical evidence.

**Keywords**——*Anodendron affine*; Apocynaceae; 3,5-diprenyl-4-hydroxybenzoic acid; nervogenic acid; 1,3-di-*O*-methyl-*myo*-inositol; dambonitol; 5-acyldambonitol; 6-acyldambonitol; $6_{glc}$-*O*-(*p*-*O*-glucosyl)nervogenoylsucrose; anodendrosin

In the preceding papers of this series, we have described the isolation of pregnanes,[2)] cardenolides,[3a)] cardenolide glycosides with double linkages between the aglycone and a sugar moiety,[1,3b)] and normal-type glycosides[3c)] from the caules and seeds of *Anodendron affine* DRUCE. This paper deals with anodendrosins A—I, glycosides of 3,5-diprenyl-4-hydroxybenzoic acid (nervogenic acid) from seeds, unripened fruits, and caules of this plant.

When the methanol extracts from the seeds and unripened fruits were partitioned between 50% MeOH and benzene, $CHCl_3$, and then *n*-BuOH, the benzene and $CHCl_3$ extracts afforded cardenolide glycosides including affinosides A,[1,3b)] M, and K.[1)]

Anodendrosins A—E were obtained as crystals (A and D) or as solids (B, C, and E) from the BuOH extract after repeated chromatography on a silica gel column. As a characteristic spectral feature, anodendrosins A—E showed two protons of the benzene ring in the proton nuclear magnetic resonance ($^1$H-NMR) spectra, anomeric carbon(s) of the sugar at $\delta$ 106, and a carbonyl carbon at $\delta$ 166 in the carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectra. The ultraviolet (UV) absorption maxima at 240—245 nm, 280 nm, and 290 nm, in addition to the evidence from the NMR spectra suggested that anodendrosins retain the phenolcarboxylic acid moiety in the molecule. The presence of a pair of prenyl groups located at symmetrical positions on a benzene ring was also indicated by two singlet peaks of six protons at $\delta$ 1.5—1.7, a broad triplet peak due to two olefinic protons ($\delta$ 5.31—5.52) in the $^1$H-NMR spectra and the corresponding five carbon signals at $\delta$ 29 (t) (C-$\alpha$), 124 (d) (C-$\beta$), 132 (s) (C-$\gamma$), 26 (q), and 18 (q).

Anodendrosin A (I), the least polar substance, has the molecular formula $C_{31}H_{46}O_{13}$, based on elementary analysis and the field desorption mass spectrum (FD-MS), and afforded a heptaacetate on acetylation. The $^{13}$C-NMR spectrum indicated carbon peaks due to one glucose unit, and the anomeric proton was observed as a doublet (7 Hz) in the $^1$H-NMR spectrum. When I was subjected to acid hydrolysis, glucose and the deglucosyl product ($C_{25}H_{38}O_9$) (I-1) were detected. Another deglucosyl product (I-2) was obtained as a solid ($C_{25}H_{36}O_8$) by enzymic hydrolysis of I. On methanolysis with $NaOCH_3$ in MeOH, I afforded the methyl ester of a phenolcarboxylic acid ($C_{24}H_{34}O_8$) (I-3) and a polyalcohol ($C_8H_{10}O_6$) (I-4), the latter of which was identified as 1,3-di-*O*-methyl-*myo*-inositol (dambonitol). In I, the

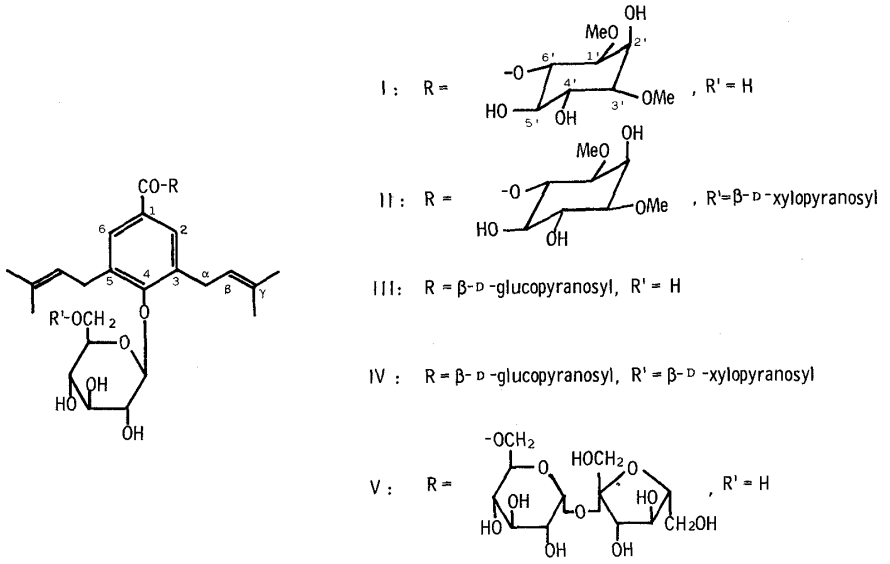


Chart 1

TABLE I. $^1$H-Chemical Shifts of I and Its Derivatives, $\delta$ (ppm) from TMS[a]

| Compd. / H | I | I-1 | I-2[b] | I-3 | I-4[c] | I-5[b] |
|---|---|---|---|---|---|---|
| 2,6-H | 8.18 (s) | 7.98 8.16 (d, 2) | 7.70 (s) | 8.05 (s) | | 7.77 (s) |
| $\alpha,\alpha'$-$H_2$ | | | 3.36 (d, 7) | 4.01 (d, 7) | | 3.38 (d, 7) |
| $\beta,\beta'$-H | 5.45 (br t, 7) | | 5.31 (br t, 7) | 5.50 (br t, 7) | | 5.33 (br t, 7) |
| $\gamma,\gamma'$-$CH_3$ | 1.58 1.68 | 1.24 1.46 | 1.76 | 1.62 1.72 | | 1.78 |
| $1_g$-H (4-*O*-glc.) | 5.40 (d, 7) | | | 5.41 (d, 7) | | |
| Others | | | | 3.80 –COOMe | | |
| R = dambonitol | | | | | | |
| 2'-H | 4.89 (br s) | 4.92 (t, 2) | 4.48 (t, 2) | | 4.57 (t, 2) | |
| 4'-H | 4.78 (t, 10) | 4.82 (t, 10) | 4.03 (t, 10) | | 3.67 (t, 10) | |
| 5'-H | | 4.28 (t, 10) | | | 3.28 (t, 10) | |
| 6'-H | 6.51 (t, 10) | 6.57 (t, 10) | 5.45 (t, 10) | | 3.67 (t, 10) | |
| –OMe | 3.44 3.67 | 3.48 3.69 | 3.44 3.53 | | 3.44 | |

*a*) Dissolved in pyridine-$d_5$ unless otherwise mentioned ($J$/Hz values in parentheses). *b*) Dissolved in $CDCl_3$. *c*) Dissolved in $D_2O$. 1'-H and 3'-H were observed at $\delta$ 3.18 (dd, $J$ = 10, 2 Hz).

phenolcarboxylic acid moiety seemed to be attached to the hydroxyl group at an asymmetrical position of dambonitol such as $C_{4'}$ or $C_{6'}$, since the signals due to the two methoxyl groups of the dambonitol were observed separately in the $^1$H-NMR spectrum of I, and glucose was considered to form a $\beta$-D-glucoside linkage with the phenolic hydroxyl group.

TABLE II. $^{13}$C-Chemical Shifts of I and Its Derivatives, $\delta$ (ppm) from TMS[a]

| Compd. / C | I | I-1 | I-2[b] | I-3 | I-5 | I-6 |
|---|---|---|---|---|---|---|
| C-1 | 128.0 | 120.5 | 121.6 | 127.0 | 129.1 | 121.7 |
| C-2 | 129.9 | 130.0[c] | 130.0 | 129.7 | 129.9 | 130.0[c] |
| C-3 | 136.4 | 131.4 | 127.1 | 136.6 | 129.1 | 131.6 |
| C-4 | 157.3 | 156.1 | 157.4 | 157.6 | 158.0 | 156.4 |
| C-5 | 136.4 | 120.5 | 127.1 | 136.6 | 129.1 | 120.7 |
| C-6 | 129.9 | 129.6[c] | 130.0 | 129.7 | 129.9 | 129.3[c] |
| C-$\alpha$ | 29.4 | 26.0 | 29.6 | 29.4 | 29.6 | 25.9 |
| C-$\beta$ | 123.8 | 44.8 | 121.4 | 123.7 | 123.0 | 44.8 |
| C-$\gamma$ | 132.4 | 69.7 | 135.0 | 132.6 | 132.8 | 69.6 |
| $\gamma$-$CH_3$ | 25.6 | 29.7 | 25.8 | 25.6 | 25.8 | 29.7 |
| | 17.9 | 29.7 | 18.0 | 17.9 | 17.8 | 29.7 |
| C-$\alpha'$ | 29.4 | 22.7 | 29.6 | 29.4 | 29.6 | 22.6 |
| C-$\beta'$ | 123.8 | 32.4 | 121.4 | 123.7 | 123.0 | 32.4 |
| C-$\gamma'$ | 132.4 | 75.1 | 135.0 | 132.6 | 132.8 | 75.3 |
| $\gamma'$-$CH_3$ | 25.6 | 26.8 | 25.8 | 25.6 | 25.8 | 26.8 |
| | 17.9 | 26.8 | 18.0 | 17.9 | 17.8 | 26.8 |
| –CO– | 166.5 | 166.8 | 167.0 | 167.0 | 169.5 | 167.0 |
| $C_g$-1[e] | 106.4 | | | 106.3 | | |
| $C_g$-2 | 75.7 | | | 75.7 | | |
| $C_g$-3 | 78.6 | | | 78.4 | | |
| $C_g$-4 | 71.7 | | | 71.9 | | |
| $C_g$-5 | 78.3 | | | 78.4 | | |
| $C_g$-6 | 62.8 | | | 62.9 | | |
| R= | dam. | dam. | dam. | –OMe | | alt. |
| C-1′ | 81.1 | 81.2 | 79.9 | 51.7 | | 66.9 |
| C-2′ | 65.7 | 65.7 | 65.9 | | | 84.0 |
| C-3′ | 83.1 | 83.2 | 81.0 | | | 70.3[d] |
| C-4′ | 73.5 | 73.6 | 72.4 | | | 81.1 |
| C-5′ | 76.0 | 75.5 | 74.3 | | | 70.4[d] |
| C-6′ | 74.7 | 74.8 | 73.5 | | | 61.3 |
| –OMe | 57.3 | 57.4 | 57.9 | | | 57.7 |
| | 57.8 | 57.8 | 58.7 | | | 58.7 |

*a*) Dissolved in pyridine-$d_5$ unless otherwise mentioned. *b*) Dissolved in $CDCl_3$. *c*, *d*) Signal assignments marked *c*) or *d*) in each column may be reversed. *e*) Nervogenic acid 4-*O*-glucoside.

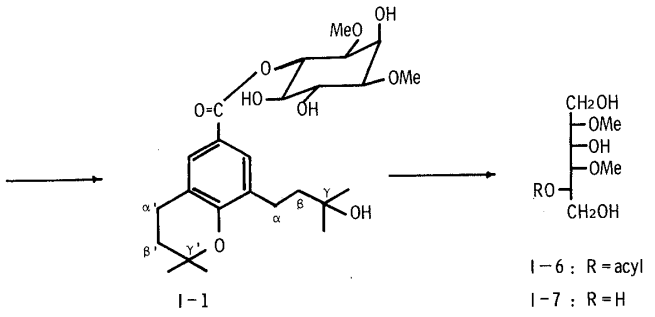


Chart 2

In order to identify the structure of the phenolcarboxylic acid, I was reacted with KOH to remove the dambonitol and was then subjected to enzymic hydrolysis. The product (I-5), mp 94—96 °C, was identified as nervogenic acid by comparisons of physical constants and spectral data with those of the compound previously obtained from *Liparis nervosa* by

Nishikawa *et al.*[4] Subsequently, I-1 was determined to be a chromane derivative formed by acid-catalyzed cyclization between the olefinic linkage of one prenyl moiety and the phenolic hydroxyl group, and introduction of $H_2O$ into the olefinic bond of the remaining prenyl moiety, based on the molecular ion ($M^+$) peak at *m/z*: 482, two doublet peaks at $\delta$ 7.98 and 8.16, coupled to each other ($J=2$ Hz), assignable to H-2 and H-6 of the benzene ring in the $^1$H-NMR spectrum, and the presence of two tertiary carbinyl carbons observed in the $^{13}$C-NMR spectrum.

On the reaction of I-1 with $HIO_4$, followed by $NaBH_4$ reduction, the product (I-6) was considered to be 2,4-di-*O*-methyl-5-acylaltritol. The deacylation of I-6 with $NaOCH_3$ in

TABLE III. $^1$H-Chemical Shifts of II—IX, $\delta$ (ppm) from TMS[a]

| H \ Compd. | II | II-2 | VII | VI | VIII[b] | IX[b] | III | IV | V |
|---|---|---|---|---|---|---|---|---|---|
| 2,6-H | 8.19 (s) | 8.05 (s) | 8.14 (s) | 8.18 (s) | 7.53 (d, 2) 7.70 (d, 2) | 7.57 (d, 2) 7.72 (d, 2) | 8.12 (s) | 8.13 (s) | 8.06 (s) |
| α-$H_2$ | | 4.03 (d, 7) | | | 3.29 (d, 7) | 3.28 (d, 7) | | | 3.92 (d, 7) |
| α′-H | | | | | 6.32 (d, 10) | 6.33 (d, 10) | | | |
| β-H | | | | | 5.25 (br t, 7) | 5.25 (br t, 7) | | | |
| β′-H | 5.45 (br t, 7) | 5.52 (br t, 7) | 5.40 (br t, 7) | 5.45 (br t, 7) | 5.62 (d, 10) | 5.62 (d, 10) | 5.43 (br t, 7) | 5.45 (br t, 7) | 5.45 (br t, 7) |
| γ-$CH_3$ | 1.59 | 1.63 | 1.54 | 1.60 | 1.73 | 1.73 | 1.56 | 1.56 | 1.62 |
| γ′-$CH_3$ | 1.69 | 1.74 | 1.67 | | 1.42 | 1.42 | 1.67 | 1.68 | 1.69 |
| $1_g$-H (4-*O*-glc.) | 5.30 (d, 7) | 5.34 (d, 7) | 5.34 (d, 8) | | | | 5.38 (d, 7) | 5.38 (d, 8) | 5.38 (d, 8) |
| Others | 4.83 (d, 7) $1_{xyl}$-H | 4.89 (d, 7) $1_{xyl}$-H 3.78 –COOMe | 4.82 (d, 7) $1_{xyl}$-H | | | | | 4.80 (d, 7) $1_{xyl}$-H | |
| R = dambonitol | | | | | | | | | |
| 2′-H | 4.88 (br s) | | | | 4.47 (t, 2) | 4.47 (t, 2) | | | |
| 5′-H | | | 5.98 (t, 10) | 6.05 (t, 10) | | 5.10 (t, 10) | | | |
| 6′-H | 6.48 (t, 10) | | | | 5.45 (t, 10) | | | | |
| –OMe | 3.45 3.65 | | 3.62 | 3.63 | 3.43 3.52 | 3.53 | | | |
| R = glucose | | | | | | | | | |
| $1_g$-H | | | | | | | 6.63 (d, 8) | 6.60 (d, 8) | |
| R = sucrose | | | | | | | | | |
| $1_g$-H (α-D-glc.) | | | | | | | | | 6.17 (d, 3) |

a) Dissolved in pyridine-$d_5$ unless otherwise mentioned ($J$/Hz values in parentheses). b) Dissolved in $CDCl_3$.

MeOH afforded 2,4-di-*O*-methylaltritol (I-7). The molecular rotation ($[M]_D$) values of I-6 and I-7 were $-5.90°$ and $-1.62°$, respectively. Since the value of L-altritol is $-5.8°$ ($[\alpha]_D$ $-3.2°$),[5] the negative values of I-6 and I-7 indicate that cleavage of the glycol linkage occurred between $C_{4'}$ and $C_{5'}$ of the dambonitol, forming L-di-*O*-methylaltritol. The location of the glucosylnervogenic acid on the dambonitol was thus determined as the $C_{6'}$-hydroxyl group of dambonitol. The structure of I was established as 1,3-di-*O*-methyl-6-*O*-(4-*O*-*β*-D-glucopyranosyl-3,5-diprenyl-4-hydroxybenzoyl)-*myo*-inositol.

Since anodendrosin C (II) was hydrolyzed to I-1, glucose and xylose with 0.5 N HCl–50% EtOH, or to dambonitol and a glycoside of methyl nervogenate (II-2) with $NaOCH_3$ in

TABLE IV. $^{13}$C-Chemical Shifts of II—IX, $\delta$ (ppm) from TMS[a]

| Compd. C | II | II-2 | VII | VI | VIII[b] | IX | III | IV | V | |
|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 128.1 | 127.1 | 128.0 | 123.2 | 120.5 | 120.8 | 126.6 | 126.6 | 127.1 | |
| C-2 | 129.9 | 129.6 | 130.0 | 130.0 | 130.8 | 131.0 | 130.2 | 130.2 | 129.7 | |
| C-3 | 136.5 | 136.6 | 136.2 | 129.1 | 129.4 | 129.3 | 136.7 | 136.7 | 136.4 | |
| C-4 | 157.2 | 157.4 | 157.1 | 157.9 | 155.6 | 154.7 | 157.9 | 157.8 | 157.5 | |
| C-5 | 136.5 | 136.6 | 136.2 | 129.1 | 122.1 | 123.9 | 136.7 | 136.7 | 136.4 | |
| C-6 | 129.9 | 129.6 | 130.0 | 130.0 | 131.6 | 131.8 | 130.2 | 130.2 | 129.7 | |
| C-$\alpha$ | 29.3 | 29.3 | 29.5 | 29.7 | 28.4 | 28.7 | 29.4 | 29.4 | 29.2 | |
| C-$\beta$ | 123.9 | 123.8 | 123.9 | 123.2 | 122.3 | 123.0 | 123.7 | 123.7 | 123.7 | |
| C-$\gamma$ | 132.4 | 132.6 | 132.2 | 132.4 | 129.4 | 129.9 | 132.5 | 132.5 | 132.7 | |
| $\gamma$-$CH_3$ | 25.7 | 25.6 | 25.6 | 25.5 | 25.7 | 25.6 | 25.6 | 25.6 | 25.6 | |
| | 18.1 | 18.1 | 18.1 | 17.8 | 17.9 | 17.8 | 18.0 | 18.1 | 18.0 | |
| C-$\alpha'$ | 29.3 | 29.3 | 29.5 | 29.7 | 126.3[c] | 126.6[c] | 29.4 | 29.4 | 29.2 | |
| C-$\beta'$ | 123.9 | 123.8 | 123.9 | 123.2 | 122.3[c] | 123.0[c] | 123.7 | 123.7 | 123.7 | |
| C-$\gamma'$ | 132.4 | 132.6 | 132.2 | 132.4 | 77.4 | 77.4 | 132.5 | 132.5 | 132.7 | |
| $\gamma'$-$CH_3$ | 25.7 | 25.6 | 25.6 | 25.5 | 28.4 | 28.1 | 25.6 | 25.6 | 25.6 | |
| | 18.1 | 18.1 | 18.1 | 17.8 | 28.4 | 28.1 | 18.0 | 18.1 | 18.0 | |
| –CO– | 166.5 | 167.0 | 166.8 | 167.2 | 167.2 | 166.6 | 165.7 | 165.7 | 166.7 | |
| $C_g$-1[d] | 106.4 | 106.2 | 106.0 | | | | 106.4 | 106.3 | 106.2 | |
| $C_g$-2 | 75.4 | 75.5 | 75.5 | | | | 75.7 | 75.4 | 75.7 | |
| $C_g$-3 | 78.2 | 78.3 | 78.3 | | | | 78.5 | 78.2 | 78.6 | |
| $C_g$-4 | 71.2 | 71.6 | 71.6 | | | | 71.7 | 71.2 | 71.9 | |
| $C_g$-5 | 77.9 | 77.6 | 77.5 | | | | 78.4 | 77.9 | 78.4 | |
| $C_g$-6 | 69.7 | 69.8 | 69.7 | | | | 62.8 | 69.6 | 63.0 | |
| $C_x$-1[e] | 105.6 | 105.3 | 105.1 | | | | | 105.6 | | |
| $C_x$-2 | 74.6 | 74.4 | 74.4 | | | | | 74.6 | | |
| $C_x$-3 | 77.1 | 77.1 | 77.3 | | | | | 77.4 | | |
| $C_x$-4 | 71.0 | 70.9 | 70.9 | | | | | 71.0 | | |
| $C_x$-5 | 67.0 | 66.6 | 66.7 | | | | | 66.9 | | |
| R= | dam. | –OMe | dam. | dam. | dam. | dam. | glc. | glc. | sucrose | |
| | | | | | | | | | fruc. | glc. |
| C-1′ | 81.1 | 51.7 | 83.3 | 83.5 | 80.2 | 83.4 | 96.5 | 96.4 | 63.0 | 93.7 |
| C-2′ | 65.6 | | 66.0 | 66.0 | 66.0 | 66.2 | 74.2 | 74.1 | 105.7 | 73.4 |
| C-3′ | 83.1 | | 83.3 | 83.5 | 81.1 | 83.4 | 79.5 | 79.5 | 80.2 | 74.8 |
| C-4′ | 73.4 | | 71.3 | 71.5 | 71.7 | 71.4 | 71.0 | 71.0 | 84.4 | 71.9 |
| C-5′ | 76.0 | | 78.7 | 78.3 | 74.6 | 78.6 | 78.8 | 78.5 | 75.9 | 71.2 |
| C-6′ | 74.6 | | 71.3 | 71.5 | 73.8 | 71.4 | 62.2 | 62.2 | 64.8 | 64.2 |
| –OMe | 57.4 | | 57.8 | 57.8 | 57.9 | 57.8 | | | | |
| | 57.8 | | | | 58.9 | | | | | |

a) Dissolved in pyridine-$d_5$ unless otherwise mentioned. b) Dissolved in $CDCl_3$. c) Signal assignments marked c) in each column may be reversed. d) Nervogenic acid 4-*O*-glucoside. e) Xylose moiety of primverose.

MeOH, II was considered to be a xyloside of I. In the $^1$H-NMR spectrum of II-2, two anomeric protons of glucose and xylose were observed at $\delta$ 5.34 (d, $J$=7 Hz) and 4.89 (d, $J$=7 Hz), respectively. Since the $^{13}$C-NMR spectrum of II showed deshielding of the $C_6$ of glucose by 6.9 ppm in comparison with that of I, II was considered to be the $\beta$-D-xylopyranosyl(1→6)-$\beta$-D-glucopyranoside (primveroside) of I-2. The structure was confirmed by the detection of methyl 2,3,4-tri-*O*-methyl-$\alpha$-D-glucopyranoside on methanolysis of II-permethylate.

Upon methanolysis, anodendrosin B (III) was cleaved to I-3 and glucose, while anodendrosin D (IV) afforded II-2 and glucose, and these results suggested that dambonitol in I and II was replaced by glucose in III and IV. The glucosidic linkages were considered to be $\beta$ from the spectral evidence that the anomeric protons of the esterified glucoside in the two compounds were observed as doublet signals at $\delta$ 6.63 and 6.60 with a coupling constant of 8 Hz in the $^1$H-NMR spectra, and that the anomeric carbon signals appeared at $\delta$ 96.5 and 96.4, respectively, in the $^{13}$C-NMR spectra.

Anodendrosin E (V) showed the most polar behavior on chromatography. On the bases of the $M^+ + Na$ peak at $m/z$ 783 in the fast atom bombardment (FAB)-MS and evidence from the $^1$H- and $^{13}$C-NMR spectra, V seemed to be composed of nervogenic acid and three hexose units. Upon hydrolysis with cellulase, V afforded nervogenic acid, while glucose and fructose were detected from the acid hydrolyzate. With $NaOCH_3$ in MeOH, V was methanolyzed to I-3 and sucrose. In the $^1$H-NMR spectrum of V, two anomeric protons were observed at $\delta$ 5.38 (d, $J$=8 Hz) and $\delta$ 6.17 (d, $J$=3 Hz), the latter of which was assigned to the anomeric proton of $\alpha$-D-glucoside in the sucrose moiety. A comparison of the $^{13}$C-NMR peaks of sucrose and those due to the sucrose moiety of V indicated that $C_6$ and $C_5$ of the glucose in the latter showed downfield and upfield shifts, respectively.[6] Therefore, the linkage of the nervogenic acid to the sucrose was determined to be at the $C_6$-hydroxyl group of the glucose moiety. When V was reacted with invertase, a less polar compound (V-1) was produced. Compound V-1 gave a positive reaction with aniline hydrogen phthalate reagent, and its structure was determined to be glucose acylated at the $C_6$-hydroxyl group by 4-*O*-glucosylnervogenic acid, on the bases of the $\alpha$- and $\beta$-anomeric proton signals at $\delta$ 5.38 (d, $J$=7 Hz) and 5.86 (d, $J$=3 Hz) in the $^1$H-NMR spectrum and all the peaks in the $^{13}$C-NMR spectrum.

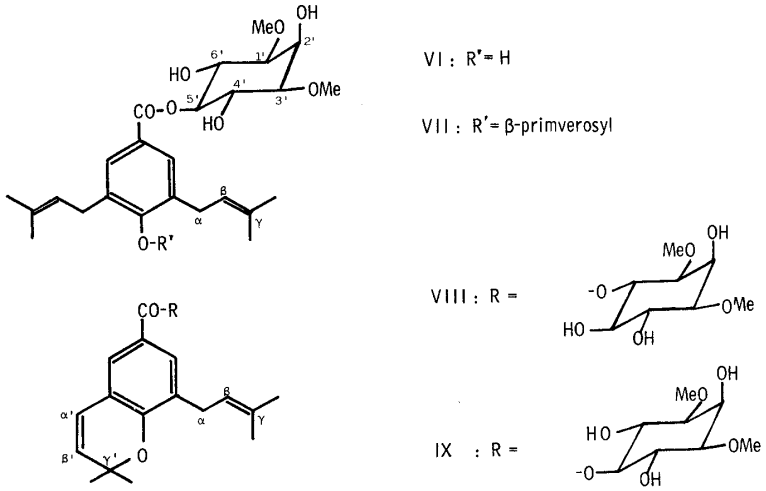


Chart 3

Homologous substances were isolated from the caules of this plant, and named anodendrosins F, G, H, and I. Anodendrosin F (VI) was found to be nervogenoyl dambonitol on the basis of spectral considerations. In the $^1$H-NMR spectrum, VI showed six protons due

to two methoxyl groups as one singlet. The nervogenic acid was, therefore, considered to be located at a hydroxyl group in a symmetrical position of dambonitol, such as $C_{2'}$ or $C_{5'}$, and the linkage was determined to be the $C_{5'}$-hydroxyl group, since the carbinyl proton on $C_{5'}$ showed a downfield shift by *ca.* 2.7 ppm in comparison with that of dambonitol.

Anodendrosin G (VII) was found to be the 4-*O*-primveroside of VI, since the presence of nervogenic acid, dambonitol, and primverose moieties was observed in the $^{13}$C-NMR spectrum, and II-2 was produced on reaction with $NaOCH_3$ in MeOH.

Anodendrosins H (VIII) and I (IX) appeared to have the same phenolcarboxylic acid. Proton signals at $\delta$ 7.53 (7.57 in IX) and 7.70 (7.72 in IX), due to the benzene ring, showed *m*-coupling of the two protons ($J=2$ Hz). One of the prenyl moieties was considered to be linked to the phenolic hydroxyl group to form a chromene derivative, on the bases of olefinic proton signals at $\delta$ 5.62 and 6.32 (6.33) coupled to each other ($J=10$ Hz), and geminal dimethyl groups as a singlet of six protons at $\delta$ 1.42. Since methoxyl protons of dambonitol were observed as two separate peaks in the spectrum of VIII, as in that of I, while a single peak was seen in the spectra of IX, VI and VII, and VIII showed a positive $[M]_D$ value (+17.1°) as did I-1 (+6.2°) and I-2 (+13.0°), the nervogenoyl residue was tentatively assigned to $C_{6'}$ of dambonitol in VIII, and to $C_{5'}$ in IX.

Dambonitol is distributed in Apocynaceae and Moraceae.[7] Shima *et al.*[8] previously reported the isolation of dambonitol, in addition to several phenol carboxylic acids having benzofuran and chromene frameworks from this plant. The present report is, however, the first to describe the isolation of dambonitol, glucose, and sucrose esterified with glycosylnervogenic acid.

## Experimental

Melting points were measured on a hot stage and are uncorrected. $^1$H- and $^{13}$C-NMR were recorded on Hitachi R-22 and JEOL FX-100 spectrometers, respectively. Samples for $^{13}$C-NMR were dissolved in pyridine-$d_5$. Chemical shifts are given in $\delta$-values referred to internal tetramethylsilane, and the following abbreviations are used; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet. MS were recorded on a JEOL D-300-FD spectrometer. UV spectra were taken in MeOH on a Shimadzu 200S double-beam spectrometer. Gas liquid chromatography (GLC) was conducted on a Shimadzu 2B mini instrument with 2% ECNSS-M on Chromosorb and $He_2$ as a carrier gas at 100 °C.

The following solvent systems were used for silica gel column chromatography and thin-layer chromatography (TLC): solv. 1, $CHCl_3$–MeOH–$H_2O$ (bottom layer); solv. 2, EtOAc–MeOH–$H_2O$ (top layer). For paper chromatographies (PC): solv. 3, *n*-BuOH–AcOH–$H_2O$ (4 : 1 : 5, top layer); solv. 4, *n*-BuOH–pyridine–$H_2O$ (3 : 2 : 1, top layer + 1 part of pyridine; multiple development). For detection of anodendrosins, 10% $H_2SO_4$ was sprayed on TLC plates and the plates were charred. For sugar, aniline hydrogen phthalate reagent was applied.

**Isolation of Anodendrosins**——a) From the Seeds: The seeds of *Anodendron affine* Druce, cultivated in the medicinal plant garden of this university, were collected in March of 1981 and 1982. The dried seeds (300 g) were homogenized with MeOH. The MeOH homogenate was filtered and the filtrate was concentrated *in vacuo*. The solution was diluted with the same volume of $H_2O$, and extracted with hexane and $CHCl_3$. The $H_2O$ layer was then concentrated, diluted with $H_2O$, and extracted with *n*-BuOH. From the $CHCl_3$ extract, cardenolide glycosides (affinosides A, K, and M) were isolated.[1] The BuOH extract (10 g) was dissolved in $H_2O$ and passed through a column of MCI gel (Mitsubishi Chem. Ind. Ltd.) and the column was eluted with 20—80% MeOH. Fractions eluted with 40—60% MeOH were chromatographed on a silica gel column with solv. 1 (7 : 2 : 1—7 : 3 : 1.2), and finally I (100 mg), III (37 mg), II (50 mg), IV (25 mg), and V (150 mg) were obtained.

b) From the Fruits: Unripened fresh fruits, harvested in September of 1981 (2 kg) were homogenized with MeOH. The MeOH percolate was worked up in the same manner as described in a). After successive column chromatographies, I (150 mg), II (50 mg), III (180 mg), and V (180 mg) were isolated.

c) From the Caules: Dried powdered caules of the plant (17 kg), harvested in March of 1982, were percolated with MeOH and the whole MeOH solution was worked up as previously described.[3] From the $CHCl_3$ extract (65.7 g), VIII was obtained as a solid showing a single spot on TLC (100 mg). VI (30 mg) and IX (930 mg) were also obtained as needles on crystallization from MeOH. The BuOH extract (164 g) was fractionated on MCI gel with $H_2O$–80% MeOH, and on a silica gel column with solv. 1 (7 : 2 : 1—7 : 3 : 1), and I (330 mg), II (550 mg), and VII (500 mg) were isolated.

**Anodendrosin A (I)**——Substance I was crystallized from MeOH to give needles, mp 140—145 °C, $[\alpha]_D^{27}$ −7.9° ($c$=1.32, MeOH). UV $\lambda_{max}^{MeOH}$ nm (ε): 242 (19000), 280 (2800), 290 (2300). *Anal.* Calcd for $C_{31}H_{46}O_{13}\cdot 1/2H_2O$: C, 58.57; H, 7.45. Found: C, 58.86; H, 7.42. FAB-MS $m/z$: 627 ($M^+$+1). Upon acetylation of I (30 mg) in 1 ml each of pyridine and $Ac_2O$ at room temperature for 24 h, I-heptaacetate was obtained and crystallized from MeOH to give needles, mp 219—220 °C. MS $m/z$: 920 ($M^+$, $C_{45}H_{60}O_{20}$).

**Hydrolysis and Methanolysis of I**——a) A 2 ml aliquot of 1 N HCl was added to the ethanolic solution of 2 ml of I (100 mg), and the mixture was refluxed for 1.5 h. The mixture was concentrated *in vacuo*, diluted with $H_2O$ and deacidified with IR-410. The solution was then extracted with *n*-BuOH. The extract was purified on a silica gel column with solv. 1 (7:1:1.5) to give I-1 (11 mg) as a solid, $[\alpha]_D^{18}$ +1.28° ($c$=2.66, MeOH). MS $m/z$: 482.250 (Calcd for $C_{25}H_{38}O_9$: 482.252). After extraction with *n*-BuOH, the $H_2O$ layer was concentrated *in vacuo*. The residue was examined by PC: $R_{glc}$ 1.00 (solv. 3), 1.00 (solv. 4). b) Enzymic hydrolysis of I was conducted with 160 mg of I with 250 mg of cellulase (Sigma Chem. Co.) in 3 ml of 10% EtOH. The mixture was shaken for 24 h at 38 °C, and then extracted with *n*-BuOH. The extract (50 mg) was purified on a silica gel column with solv. 1 (7:1:2) to give a solid (I-2), $[\alpha]_D^{27}$ +2.8° ($c$=0.76, MeOH). MS $m/z$: 464.240 ($M^+$, Calcd for $C_{25}H_{36}O_8$: 464.241). Substance I-2 was acetylated with pyridine and $Ac_2O$ to give I-2 acetate as a solid. c) Substance I (60 mg) was dissolved in 2 ml of $NaOCH_3$ in MeOH (250 mg Na/50 ml MeOH) and the solution was allowed to stand at room temperature for 2 h, then diluted with MeOH, and neutralized with IR-120B. The methanolic solution was concentrated *in vacuo*. After dilution with $H_2O$, the solution was extracted with *n*-BuOH. The BuOH extract was purified on a silica gel column with solv. 1 (7:2:2) to give a solid (I-3) (23 mg). FD-MS $m/z$: 450 ($M^+$, $C_{24}H_{34}O_8$). After BuOH extraction, the $H_2O$ layer was passed through a charcoal column. The effluent with 5% EtOH was concentrated to dryness *in vacuo*, and the residue was crystallized from MeOH to give needles mp 212—214 °C, which showed no depression on admixture with authentic dambonitol (mp 212—213 °C). d) Anodendrosin A (200 mg) was refluxed with 5 ml of 5% KOH in EtOH, and the mixture was neutralized with IR-120B, concentrated *in vacuo*, diluted with $H_2O$, and extracted wtih *n*-BuOH. Without purification, the BuOH extract was dissolved in 2 ml of $H_2O$, and shaken with 200 mg of cellulase for 24 h at 38 °C. The mixture was extracted with *n*-BuOH, and the BuOH extract was purified on a silica gel column with solv. 1 (7:1:0.8). The effluent giving a single spot on TLC (I-5) was crystallized from hexane to give colorless needles (16 mg), mp 94—96 °C. MS $m/z$: 274.156 ($M^+$, Calcd for $C_{17}H_{22}O_3$: 274.157).

**2,4-Di-*O*-methyl-L-altritol from I-1**——A methanolic solution of I-1 (260 mg/10 ml) was treated with 2 ml of $HIO_4$ (400 mg) in $H_2O$, and the mixture was allowed to stand for 24 h at room temperature. The mixture was then extracted with $CHCl_3$ and the $CHCl_3$ extract was dissolved in 10 ml of MeOH and reacted with 100 mg of $NaBH_4$ for 30 min. The mixture was diluted with $H_2O$ and extracted with *n*-BuOH. The BuOH extract was purified on a silica gel column with solv. 1 (7:1:2) to give I-6 as a solid (80 mg), $[\alpha]_D^{18}$ −1.22° ($c$=1.8, MeOH), $[M]_D$ −5.90°. I-6 was dissolved in 1 ml of $NaOCH_3$ in MeOH (50 mg Na/10 ml MeOH) and the solution was allowed to stand for 1.5 h. The residue was neutralized with IR-120B and the methanolic solution was concentrated to dryness *in vacuo*. The residue was passed through an MCI gel column. The effluent with $H_2O$ yielded I-7 as a solid (26 mg), $[\alpha]_D^{18}$ −0.77° ($c$=1.30, MeOH), $[M]_D$ −1.62°. MS $m/z$: 211 ($M^+$+1, $C_8H_{18}O_6$). Upon usual acetylation with pyridine and $Ac_2O$, tetraacetate of I-7 was obtained as a solid. MS $m/z$: 379 ($M^+$+1, $C_{16}H_{26}O_{10}$).

**Anodendrosin C (II)**——Anodendrosin C was obtained as a solid, $[\alpha]_D^{27}$ −37.8° ($c$=0.81, MeOH). UV $\lambda_{max}^{MeOH}$ nm (ε): 242 (14800), 280 (sh, 2400), 290 (sh, 1800). FD-MS $m/z$: 781 ($M^+$+Na, $C_{36}H_{54}O_{17}$). After hydrolysis of II (230 mg) with 0.5 N $H_2SO_4$–50% EtOH (6 ml), the EtOH was evaporated off *in vacuo*. The residue was diluted with $H_2O$, and extracted with *n*-BuOH. The BuOH extract was purified on a silica gel column with solv. 1 (7:1:1.5). From the effluent, 35 mg of I-1 was obtained. The $H_2O$ layer was neutralized with IR-410 and the $H_2O$ was evaporated off *in vacuo*. From the residue, glucose and xylose were detected on PC: $R_{glc}$ 1.00, 1.36 (solv. 3, xylose: 1.36), $R_{glc}$: 0.99, 1.23 (solv. 4, xylose: 1.22). The alkaline hydrolysis of II was conducted with 150 mg of II and 4 ml of $NaOCH_3$ in MeOH (the same concentration as above) for 2 h at room temperature, and the mixture was neutralized with IR-120B. The solution was concentrated *in vacuo*, diluted with $H_2O$, and extracted with *n*-BuOH. The BuOH extract was purified on a silica gel column with solv. 1 (7:2:1) and crystallized from AcOEt–MeOH to give 60 mg of prisms (II-2), mp 168—174 °C. FAB-MS $m/z$: 605 ($M^+$+Na, $C_{29}H_{42}O_{12}$). *Anal.* Calcd for $C_{29}H_{42}O_{12}$: C, 59.78; H, 7.27. Found: C, 59.43; H, 7.51. The $H_2O$ layer was passed through a charcoal column and eluted with 5% EtOH. The product was crystallized from MeOH to give dambonitol as crystals, mp 211—213 °C.

**Methylation of II and Identification of Methyl Sugars**——The methylation of II was conducted according to Kuhn's method[9] with MeI and $Ag_2O$ in dimethylformamide. The crude permethylate of II was purified by silica gel column chromatography with solv. 1 (7:1:1). The permethylate, showing a homogeneous spot on TLC, was refluxed with 1 N HCl–MeOH for 40 min, and the solution was deacidified with IR-410. The solution was concentrated *in vacuo* and the residue was purified on a silica gel column with benzene–acetone (10:1). The effluent was subjected to GC: $t_R$ 0.6 min, 4.7 min (Me permethyl-α-D-xyloside, 0.6 min; Me 2,3,4-tri-*O*-methyl-α-D-glucoside, 4.6 min; Me 2,3,6-tri-*O*-methyl-α-D-glucoside, 6.7 min; Me 2,4,6-tri-*O*-methyl-α-D-glucoside, 7.5 min; Me 3,4,6-tri-*O*-methyl-α-D-glucoside, 3.7 min).

**Anodendrosin B (III)**——Substance III was obtained as a solid, $[\alpha]_D^{16}$ −15.0° ($c$=2.05, MeOH). FAB-MS: 621

($M^+$+Na, $C_{29}H_{42}O_{13}$). UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 247 (14000), 280 (sh, 4000), 290 (2900). On reaction with $NaOCH_3$, followed by neutralization and BuOH extraction, I-3 and glucose were detected from the BuOH extract (TLC, solv. 1, 7:2:1 and solv. 2, 9:1:0.1), and the $H_2O$ layer (PC, solv. 3), respectively.

**Anodendrosin D (IV)**——Substance IV was crystallized from MeOH to give needles, mp 180—185 °C, $[\alpha]_D^{27}$ −20.2° ($c$=0.57, MeOH). *Anal.* Calcd for $C_{34}H_{50}O_{17}\cdot 3H_2O$: C, 52.04; H, 7.19. Found: C, 51.98; H, 6.59. FD-MS *m/z*: 753 ($M^+$+Na, $C_{34}H_{50}O_{17}$). UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 243 (13500), 290 (sh, 2200). Upon the methanolysis of IV (15 mg) with $NaOCH_3$ in MeOH for 5 h at room temperature, followed by the usual procedure, II-2 and glucose were detected from the BuOH and $H_2O$ layers, on TLC and PC, respectively.

**Anodendrosin E (V)**——Substance V was obtained as a solid, $[\alpha]_D^{27}$ +4.28° ($c$=11.95, MeOH). FAB-MS *m/z*: 783 ($M^+$+Na, $C_{35}H_{52}O_{18}$), UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 242 (15900), 280 (sh, 2300).

**Hydrolysis of V**——a) The solution of V (10 mg) in 2 ml of 0.5 N HCl–50% EtOH was refluxed for 2 h, and worked up as described above. From the $H_2O$ layer, glucose and fructose were detected on PC, $R_{glc}$: 1.00, 1.55 (solv. 3, glucose: 1.00, fructose: 1.17). b) A mixture of V (60 mg) with 3 ml of $NaOCH_3$ in MeOH was allowed to stand for 5 h at room temperature, then worked up as described above. From the BuOH extract, I-3 was detected on TLC (solv. 1, 7:2:1; solv. 2, 9:2:0.1). After BuOH extraction, the $H_2O$ layer was concentrated to dryness *in vacuo*, and the residue was chromatographed on a charcoal column. From the effluent with $H_2O$, prisms were obtained, mp 185—190 °C, and identified as sucrose by comparison with an authentic sample. c) A solution of V (80 mg) in 5 ml of $H_2O$ was treated with 1.5 ml of invertase for 3 h at 38 °C, with shaking. The mixture was extracted with *n*-BuOH and the BuOH extract was purified on a silica gel column with solv. 1 (7:2:1) to give a homogeneous solid (V-1), FD-MS *m/z*: 621 ($M^+$+Na, $C_{29}H_{42}O_{13}$). After spraying of the TLC plate with the aniline hydrogen phthalate reagent, followed by heating, V-1 showed a brown color.

**Anodendrosin H (VIII)**——Substance VIII was obtained as a solid, $[\alpha]_D^{18}$ +3.7° ($c$=0.50, MeOH). MS *m/z*: 462.2259 (Calcd for $C_{25}H_{34}O_8$: 462.2254).

**Anodendrosin I (IX)**——Substance IX was crystallized from MeOH to give prisms, mp 194—195 °C. MS *m/z*: 462.2261 (Calcd for $C_{25}H_{34}O_8$: 462.2254). UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 243 (24000), 280 (3600), 305 (2300), 318 (1200).

**Anodendrosin F (VI)**——Substance VI was crystallized from MeOH to give needles, mp 130—135 °C. FD-MS *m/z*: 464.2377 (Calcd for $C_{25}H_{36}O_8$: 464.2410). UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 245 (15000), 280 (sh, 4400).

**Anodendrosin G (VII)**——Substance VII was crystallized from MeOH to give prisms, mp 176—184 °C, $[\alpha]_D^{25}$ −36.4° ($c$=0.67, MeOH). *Anal.* Calcd for $C_{36}H_{54}O_{17}\cdot 1/2H_2O$: C, 56.32; H, 7.22. Found: C, 56.24; H, 7.23. The methanolysis of VII (90 mg) with $NaOCH_3$ in MeOH was conducted in the usual manner, and II-2 (40 mg) was obtained as needles, mp 170—174 °C, after purification on a column and crystallization from MeOH. From the $H_2O$ layer, dambonitol was obtained as crystals, mp 211—214 °C, after charcoal column chromatography followed by crystallization from MeOH.

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