Journal of Natural Products
Vol. 53, No. 2, pp. 303-308, Mar-Apr 1990

NEW BENZOIC ESTERS OF LOGANIN AND 6β-HYDROXYLOGANIN FROM *NYCTANTHES ARBOR-TRISTIS*[1]

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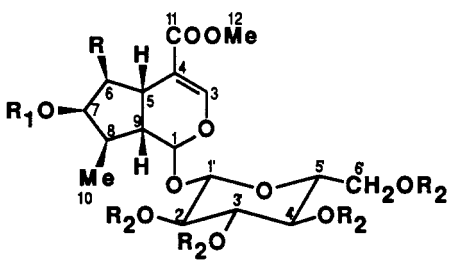
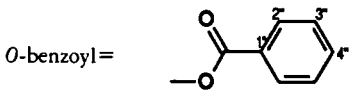
ABSTRACT.—Three new iridoid glycosides, arborsides A [**1**], B [**2**], and C [**3**], were isolated from the leaves of *Nyctanthes arbor-tristis*. The absolute structures were determined by spectral data (uv, ir, ms, nmr) and chemical correlation of the products of alkaline hydrolysis of arborside A tetraacetate, arborside B tetraacetate (on subsequent methylation and acetylation), and arborside C with known iridoids 6β-hydroxyloganin hexaacetate, loganin pentaacetate, and 6β-hydroxyloganin, respectively.

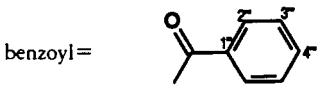
Nyctanthes arbor-tristis L. is a large shrub placed in the family Verbenaceae by Bentham and Hooker (1). The plant is cultivated as a garden plant throughout India and is found wild in the forests of Madhya Pradesh and in the sub-Himalayan regions. Previous work (2,3) reported that the leaves of the plant exhibit a wide variety of potent and interesting biological activities. The juice of the leaves is recommended as a purgative, a vermifuge for expelling roundworms and threadworms, in chronic fever, a specific for obstinate sciatica, and as an antimalarial remedy (4). The leaves are antibilious, expectorant, and anthelmintic and are used as a diaphoretic, diuretic, cholagogue, and laxative.

Previous chemical work on the seeds of *N. arbor-tristis* revealed the presence of three iridoid glucosides, arbortristosides A, B, and C (5,6). Investigation of the leaves of this plant has now resulted in the isolation of three new iridoid glucosides, arborsides A [**1**], B [**2**], and C [**3**].

The $CHCl_3$ fraction of a 50% EtOH extract of *N. arbor-tristis* contained a complex mixture of iridoid glucosides as shown by tlc analysis. Gross cc of this mixture on Si gel yielded fraction A. This fraction on rechromatography resulted in a subfraction containing a mixture of compounds **1** and **2** and pure compound **3**. This subfraction was acetylated with Ac_2O and pyridine, and the resulting acetates were subjected to preparative tlc to afford arborsides A and B as their tetraacetates **4** and **5**, respectively.







1 R=*O*-benzoyl, R_1=benzoyl, R_2=H
2 R=R_2=H, R_1=benzoyl
3 R=OH, R_1=benzoyl, R_2=H
4 R=*O*-benzoyl, R_1=benzoyl, R_2=Ac
5 R=H, R_1=benzoyl, R_2=Ac
6 R=OAc, R_1=benzoyl, R_2=Ac
7 R=OAc, R_1=R_2=Ac
8 R=H, R_1=R_2=Ac
9 R=R_1=OH, R_2=H

[1]CDRI Communication No. 4242.

The uv spectra of **4**, **5**, and **3** exhibited typical absorptions for iridoid glucosides (7) with a C-4 methoxy carbonyl group (λ max at 229, 227 and 230 nm) and benzoyl moiety (λ max at 281 and 280 nm). The compounds gave a positive vanillin reaction, suggesting a probable iridoid structure.

Compound **4**, $C_{39}H_{42}O_{17}$, was obtained as white needles, mp 138°, exhibiting ir absorption bands at 1740 (ester group), 1640 (double bond), and 1230 cm^{-1} (acetoxy group); fdms of **4** showed a molecular ion peak at *m/z* 782 (740, 698, 656, and 614) and successive losses of 42 mass units ($CH_2=C=O$); it also showed a significant peak at 331 which could be assigned to the loss of acetylated sugar from the molecule. Evidence for the presence and location of the benzoyl moiety was obtained from eims and the ^{1}H-nmr spectrum. In the eims (70 eV), peaks at *m/z* 122 and 105 were attributed to benzoic acid and a benzoyl moiety.

Compound **5**, $C_{32}H_{38}O_{15}$, from C_6H_6/Me_2CO is an amorphous powder with the ir spectrum showing characteristic absorption for iridoids (1730 and 1650 cm^{-1}). In fdms, the molecular ion peak appeared at *m/z* 662. Comparison of the ^{1}H-nmr spectra of **4** and **5** with those of authentic samples of 6β-hydroxyloganin hexaacetate and loganin pentaacetate showed a close structural relationship in the iridoid signals (Table 1). Signals in the ^{1}H-nmr spectrum of **4** pertaining to the benzoic acid units acylating the iridoid at positions C-6 and C-7 were found at δ 7.28–7.98 ppm integrating for 10 protons, and signals observed at δ 7.40–7.95 were attributed to a benzoyl moiety acylating **5** at C-7, showing that chemical shift variations noticed in both compounds were induced by the acyl residues. In addition the ^{1}H-nmr spectra (Table 1) of **4** and **5** showed signals for methyl groups at δ 1.15 (d, $J=7.5$ Hz) and 0.85 (d, $J=8.0$ Hz), olefinic protons at δ 7.26 (s) and 7.24 (s), acetal protons at δ 5.38 (d) and 5.14 (d), axial protons due to the glucose moiety at δ 3.78–5.26 and δ 4.20–5.58, and acetyl group signals at δ 1.90–2.11 and δ 1.93–2.14, respectively.

Compound **3** was isolated as a white crystalline substance, mp 210–212°, and had the composition $C_{24}H_{30}O_{12}$ on the basis of elemental analysis and fdms data ($[M]^+$ peak at *m/z* 510). The difference in the R_f values [0.55 for **3** against 0.25 for 6β-hydroxyloganin] suggested the presence of an ester group in addition to the basic iridoid skeleton. Compound **3** on acid treatment afforded glucose (1 mol) and black products arising from the decomposition of the aglucone (8). The assignments of ^{1}H-nmr (Table 1) and ^{13}C-nmr (Table 2) spectral data of **3** could be rationalized into two parts. Many signals were readily assignable to a 6β-hydroxyloganin moiety with the probability of acylation at position 7; all chemical shifts and coupling constant values were very similar, apart from differences due to acylation effects.

A triplet at δ 5.41 ppm ($J=5.0$ Hz) in the ^{1}H-nmr spectrum of **3** is assigned to H-7 as this proton is equally coupled to H-6α and H-8α (6,9).

Reaction of **3** with Ac_2O in pyridine at room temperature gave the pentaacetyl derivative **6**, the ^{1}H- and ^{13}C-nmr spectra of which confirmed previous assignments. The constancy of the triplet at H-7 reinforced the assignment of the acylation site at position 7 (6). On acetylation, the multiplet due to H-6 (δ 3.24–3.78) showed a downfield shift of approximately 2 ppm (δ 5.37), from which we conclude that a hydroxyl group is present at C-6.

The stereochemistry at various centers of compound **3** was determined by ^{13}C nmr and correlation of the alkaline hydrolysis product with 6β-hydroxyloganin (10). The identity of the sugar moiety was confirmed by the ^{13}C-nmr data exhibiting characteristic resonances at δ 99.4, 73.9, 76.8, 70.9, 77.4, and 62.0 ppm. The sugar signals suggested that in compound **3** the glycosidic moiety was glucose in a β-configuration at C-1′. Other assignments were in accordance with values reported for the iridoids (11,12).

TABLE 1. ^{1}H-nmr Chemical Shift Assignments of Compounds **3–9** (chemical shift values in ppm, coupling constants in Hz).

Proton	Compound						
	4[a] (200 MHz)	**7**[a] (80 MHz)	**5**[a] (80 MHz)	**8**[a] (90 MHz)	**3**[a] (200 MHz)	**6**[b] (200 MHz)	**9**[b] (90 MHz)
H-1	5.38 (d, J = 4.0)	5.24 (m)	5.14 (d, J = 7.0)	5.14 (d, J = 7.5)	5.00 (d, J = 7.5)	5.37 (m)	5.40 (d, J = 5.0)
H-3	7.26 (s)	7.34 (s)	7.24 (s)	7.18 (d, J = 0.5)	7.26 (d, J = 1.8)	7.38 (d, J = 1.8)	7.54 (s)
H-5	3.24 (dd, J = 4.0 and 9.0)	2.98 (dd, J = 6.0 and 9.0)	3.11 (m)	2.96 (m)	3.03 (dt, J = 1.2 and 8.0)	3.06 (dd, J = 3.4 and 8.6)	2.80 (m)
H-6	5.56 (t, J = 6.0)	5.24 (m)	H-6α 1.75 (m) H-6β 2.65 (m)	1.80–2.30 (m)	3.24–3.78 (m)	5.37 (m)	3.91 (m, 2H)
H-7	5.65 (t, J = 4.0)	4.18 (t, J = 4.5)	5.34 (brt, J = 4.0 and 7.0)	4.60–5.10 (m)	5.41 (t, J = 5.0)	5.46 (t, J = 5.4)	
H-8	2.32 (m)	2.35 (m)	2.30 (m)	1.80–2.30 (m)	2.20 (m)	2.20 (m)	1.90 (m)
H-9	2.72 (dt, J = 2.0 and 10.0)	2.52 (t, J = 10.0)	2.65 (m)	1.80–2.30 (m)	2.20 (m)	2.62 (dt, J = 1.8 and 9.4)	2.28 (m)
H-10	1.15 (d, J = 7.5)	1.07 (d, J = 7.0)	0.85 (d, J = 8.0)	0.98 (d, J = 7.0)	1.01 (d, J = 7.0)	1.10 (d, J = 7.0)	1.19 (d, J = 7.0)
H-12	3.60 (s)	3.67 (s)	3.58 (s)	3.62 (s)	3.67 (s)	3.67 (s)	3.82 (s)
H-1′	4.88 (d, J = 8.0)		4.92 (d, J = 8.0)		4.62 (d, J = 8.0)	4.85 (d, J = 7.9)	4.62 (d, J = 7.0)
H-2′	5.01 (t, J = 9.0)[c]		—			4.96 (t, J = 8.3)[c]	
H-3′	5.26 (t, J = 9.0)	4.5–5.5 (m)	4.50–5.58 (m)		3.24–3.78 (m)	5.24 (t, J = 8.0)	
H-4′	5.11 (t, J = 8.0)[c]		—	4.60–5.10 (m)		5.10 (t, J = 8.0)[c]	3.68–3.90 (m)
H-5′	3.78 (m)					3.75 (m)	
H-6′a	4.16 (m)		4.20 (m)	4.05 (dd, J = 2.5 and 12.5)		4.14 (m)	
H-6′b	4.34 (dd, J = 4.2 and 11.0)		4.44 (dd, J = 4.0 and 11.0)	4.26 (dd, J = 2.5 and 12.5)	3.92 (dd, J = 2.5 and 12.0)	4.32 (dd, J = 2.5 and 11.8)	
H-2″ and 6″	7.28–7.98 (m) for 10H		7.40–7.95 (m) 5H	—	7.95–8.02 (m)	7.96–8.04 (m)	
H-3″ and 4″ and 5″					7.31–7.54 (m)	7.40–7.64 (m)	—
Acetates	1.90, 1.99, 2.03, 2.11 (s)	1.92, 2.04, 2.06, 2.10, 2.12 (s)	1.93, 2.02, 2.05, 2.14 (s)	1.85–2.04 (s)	—	1.90, 1.97, 2.00, 2.03, 2.10 (s)	—

[a]In $CDCl_3$.
[b]In DMSO-d_6.
[c]Values are interchangeable.

TABLE 2. ^{13}C-nmr Chemical Shift Assignments of Compounds **4**, **3**, **6**, and **9** (values in ppm, TMS as internal standard).

Carbon	Compound			
	4[a] (100.13 MHz)	**3**[b] (20 MHz)	**6**[a] (50.30 MHz)	**9**[b] (100.13 MHz)
C-1	94.56	95.9	94.06	95.42
C-3	151.03	153.0	150.66	151.38
C-4	110.03	109.4	110.25	109.33
C-5	36.20	37.2	36.24	37.57
C-6	76.99[c]	77.4[c]	75.38[c]	77.12[c]
C-7	76.67[c]	78.0[c]	75.69[c]	72.58[c]
C-8	35.75	36.1	35.44	36.60
C-9	45.43	45.8	44.91	44.04
C-10	13.81	15.8	13.35	13.96
C-11	166.39	168.2	166.40	167.34
C-12	51.44	52.1	51.50	50.92
C-1′	96.11	99.4	95.81	98.50
C-2′	70.69	73.9	70.47	73.03
C-3′	72.57	76.8	72.38	77.74
C-4′	68.36	70.9	68.07	70.04
C-5′	72.42	77.4[c]	72.26	76.68
C-6′	61.80	62.0	61.63	61.08
C-1″ and 1‴	129.92	131.0	133.22	—
C-2″ and 6″	129.75[d]	130.0	129.62	—
C-3″ and 5″	128.30[e]	129.5	128.49	—
C-4″ and 4‴	133.02[f] 132.87[f]	134.0	[h]	—
C-2‴ and 6‴	129.66[d]	—	—	—
C-3‴ and 5‴	128.24[e]	—	—	—
C-7″	165.26[g]	166.5	165.75	—
C-7‴	165.68[g]	—	—	—
5 × O ‖ C − CH$_3$	169.02, 169.28, 169.99, 170.42	—	169.09, 169.38, 169.70, 170.04, 170.57	—
5 × OCOCH$_3$	20.09, 20.47, 20.62	—	20.74, 20.69, 20.57, 20.12	

[a]In $CDCl_3$.
[b]In DMSO-d_6.
[c–g]Values in the same column with same superscript may be interchanged.
[h]See Imakura *et al.* (12).

These conclusions were also confirmed by treatment of these compounds in alkaline conditions, affording products which were identified as 6β-hydroxyloganin hexaacetate [**7**], loganin pentaacetate [**8**], and 6β-hydroxyloganin [**9**] by direct comparison with authentic samples (co-tlc, superimposable ir, mmp).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps uncorrected; eims 70 eV; fdms JEOL JMS-OSIG 2 instrument; uv Lambda-15 (Perkin-Elmer); ^{1}H nmr 80, 90, and 200 MHz; ^{13}C nmr 50.309 MHz with TMS as an internal standard; cc Si gel 60–120, Sisco; tlc and plc Si gel 60, Sisco. Spots and bands were visualized by I_2 vapor, and spraying reagents for iridoids were (a) 1% ceric sulfate in 2 N H_2SO_4, and (b) vanillin and H_2SO_4 (1%) in 100 ml EtOH followed by heating at 100–110° for 5–10 min.

PLANT MATERIAL.—Leaves of *N. arbor-tristis* were collected from the Mohand forest of district Dehradoon, Uttar Pradesh, India and identified by Dr. B.S. Aswal. A voucher specimen (11375 CDRI) has been deposited in the herbarium of Medicinal Plants of Central Drug Research Institute, Lucknow.

ISOLATION PROCEDURE.—The leaves of *N. arbor-tristis* (20 kg) were exhaustively extracted with EtOH (3 × 15 liters) at room temperature. The extract was concentrated in vacuo below 50° to give a dark green mass (752 g). The EtOH extract on subsequent fractionation with C_6H_{14}, $CHCl_3$, and *n*-BuOH gave C_6H_{14}-soluble (56 g), $CHCl_3$-soluble (304 g), *n*-BuOH-soluble (292 g), and H_2O-soluble (80 g) fractions.

Cc of the $CHCl_3$ extract (60 g) was carried out on a Si gel (1.5 kg) column by eluting with $CHCl_3$/MeOH, with increasing MeOH content. Fraction A was obtained by eluting with $CHCl_3$-MeOH (9:1), which on rechromatography over Si gel (1.5 kg) and elution with C_6H_{14}/EtOAc with increasing EtOAc content yielded an inseparable mixture of compounds **1** and **2**. Compound **3** was obtained directly from fraction A on elution with 70% EtOAc/C_6H_{14}, as white needles (250 mg), mp 210–212° (EtOAc/C_6H_{14}). The mixture containing compounds **1** and **2** was acetylated with Ac_2O/pyridine at room temperature. The acetylated product on usual workup and separation by preparative tlc afforded pure compounds **1** and **2** as their acetate derivatives **4** and **5**, respectively [C_6H_6-Me_2CO (9:1), double run].

ARBORSIDE A TETRAACETATE [**4**].—Compound **4** (160 mg) was isolated as white needles: mp 138°, $[\alpha]^{28}D$ +9.7° (c = 1.9, $CHCl_3$); uv λ max (MeOH) 281, 229 nm; ir ν max (KBr) 2980, 1740, 1640, 1460, 1390, 1295, 1260, 1230, 1100, 765, 720 cm^{-1}; eims *m/z* 435, [M − glu(OAc)$_4$ − OH]$^+$ 434, 406, 404, 403, 372, 366, 350, [glu(OAc)$_4$]$^+$ 331, 228, 208, 190, 165, 160, 133, 122 (benzoic acid), 105 (base peak, benzoyl moiety), 77, 60, 42; fdms *m/z* [M]$^+$ 782, 740, 712, 698, 662, 656, 614, 526, 472, 450, 348, 331, 323; ^{1}H-nmr (200 MHz) see Table 1. *Anal.* calcd for $C_{39}H_{42}O_{17}$, C 59.68, H 5.43; found C 59.84, H 5.37.

ARBORSIDE B TETRAACETATE [**5**].—Compound **5** (140 mg) was isolated as an amorphous powder: $[\alpha]^{28}D$ +7.3° (c = 0.3, $CHCl_3$); uv λ max (MeOH) 280, 227 nm; ir ν max (KBr) 2990, 1730, 1650, 1430, 1390, 1230, 1060, 770 cm^{-1}; eims *m/z* 459, 458, 430, 420, 410, 368, 345, 327, 322, 317, 313, 303, 300, 278, 257, 255, 192, 185, 162, 149, 139, 133, 122, 105, 96, 84, 82, 70, 63, 55; fdms *m/z* [M]$^+$ 662; ^{1}H nmr see Table 1. *Anal.* calcd for $C_{32}H_{38}O_{15}$, C 58.00, H 5.74; found C 58.39, H 5.59.

ARBORSIDE C [**3**].—Compound **3** (250 mg) was isolated as white needles: mp 210–212° (EtOAc/C_6H_{14}), $[\alpha]^{28}D$ −102° (c = 1.0, MeOH); uv λ max (MeOH) 280, 230 nm; ir ν max (KBr) 3520, 3320, 1700, 1625, 1622, 1440, 1280, 1180, 1080, 1020, 950, 865, 710 cm^{-1}; eims *m/z* [M − glucose]$^+$ 348, 331, 226, 209, 168, 148, 139, 122, 105, 77; fdms *m/z* [M]$^+$ 510; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{24}H_{30}O_{12}$, C 56.88, H 6.11; found C 56.47, H 5.88.

ARBORSIDE C PENTAACETATE [**6**].—Compound **6** (20 mg) was isolated as colorless needles: mp 145–146° (MeOH/$CHCl_3$), $[\alpha]^{28}D$ −78° (c = 1.0, MeOH); uv λ max (MeOH) 229 nm; ir ν max (KBr) 2975, 1760, 1730, 1640, 1620, 1510, 1445, 1380, 1220 (acetoxy), 1070, 1040 cm^{-1}; eims *m/z* [M − glu(OAc)$_4$]$^+$ 390, 373, 268, 331, 210, 168, 139, 122, 105 (benzoyl group); fdms *m/z* [M]$^+$ 720, [glu(OAc)$_4$]$^+$ 331; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

ALKALINE HYDROLYSIS.—Each compound (80 mg) was dissolved in MeOH and 5 ml 2% KOH. The reaction mixture was refluxed gently for 3 h, concentrated, and neutralized with HCl and extracted with EtOAc and *n*-BuOH successively. The EtOAc layer was concentrated to give a residue which on purification by preparative tlc was identified as benzoic acid, by comparison with an authentic sample by tlc, mmp, superimposable ir, and ^{1}H nmr.

The *n*-BuOH layer was concentrated in vacuo, dissolved in MeOH, and treated with CH_2N_2 at 0° for 40 h.

ACETYLATED DERIVATIVES.—The methylated products of **4** and **5** were acetylated with Ac_2O/pyridine at room temperature to give solid residues **7** (identified as 6β-hydroxyloganin hexaacetate) and **8** (identified as loganin pentaacetate), respectively (ms, ^{1}H nmr, superimposable ir, co-tlc). Compound **7** (10 mg) was obtained as colorless crystals (EtOAc/C_6H_{14}): mp 109–111°; ir ν max (KBr) 2960, 1760, 1715, 1645, 1450, 1380, 1300, 1240, 1180, 1085, 1040, 960, 915, 870, 805, 780, 770, 700 cm^{-1}; ^{1}H nmr see Table 1. *Anal.* calcd for $C_{29}H_{38}O_{17}$, C 52.75, H 5.47; found C 52.88, H 5.47.

Compound **8** (12 mg) was obtained as colorless needles (EtOAc/C_6H_{14}): mp 140°; ir ν max (KBr) 2950, 1760 (acetate), 1720 (-COOMe), 1650 (enol ether) cm^{-1}; ^{1}H nmr see Table 1. *Anal.* calcd for $C_{27}H_{36}O_{15}$, C 53.67, H 6.22; found C 54.00, H 6.00.

Solvent was removed from the reaction product, and it was recrystallized from EtOH as white shining needles and identified as 6β-hydroxyloganin [**9**] by comparison with an authentic sample by tlc and spectral data.

Compound **9** (18 mg) was obtained as white shining needles: mp 220–222°; ir ν max (KBr) 3350 (OH), 2990, 1705 (C=O, ester), 1650 (C=C), 1300, 1210, 1190, 1090, 890, 760 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{17}H_{26}O_{11}$, C 50.39, H 6.23; found C 50.20, H 6.40.

ACID HYDROLYSIS.—Compounds **4**, **5**, and **3** (5 mg each), dissolved in 1 N H_2SO_4 (10 ml), were refluxed for 4 h. The black degradation products were removed by filtration. The acid solution was neutralized with strong anion exchange resin IR 410 CO_3^{--} to give a viscous mass identical with glucose, identified by direct comparison with an authentic sample on paper chromatography.

ACKNOWLEDGMENTS

We thank Dr. B.S. Aswal, Botany Department, CDRI, for collection and identification of plant material and Mr. A.K. Srivastava for his technical assistance.

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Received 28 June 1989