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ARBORTRISTOSIDE A AND B, TWO IRIDOID GLUCOSIDES FROM *NYCTANTHES ARBOR-TRISTIS*

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Key Word Index—*Nyctanthes arbor-tristis*; Oleaceae; iridoid glucosides; arbortristosides A and B.

Abstract—Two new iridoid glucosides, arbortristoside A and B have been isolated from the seeds of *Nyctanthes arbor-tristis*. The structures of the two new compounds were determined by spectroscopic methods and chemical reactions.

INTRODUCTION

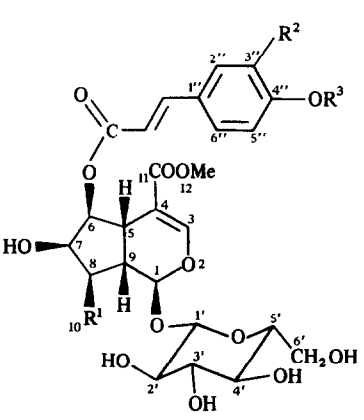
Nyctanthes arbor-tristis L. is a traditional drug used in the treatment of chronic fevers and rheumatism. It is also used as a helminthocide, and a liver and nerve tonic. The systematic position of *Nyctanthes arbor-tristis* has been the subject of discussion. It was placed in the Oleaceae by Bentham and Hooker [1]. However, a verbenaceous affinity for Nyctanthes was suggested by Stant [2]. Airy Shaw assigned the genus to the sub-family Nyctanthoideae in the family Verbenaceae [3]. Das and Rao have suggested a better assignment to the family Oleaceae rather than Verbenaceae [4]. Chemical examination of the seeds of this plant resulted in the isolation of two new iridoids designated as arbortristoside A and B. Earlier workers have reported the isolation of nyctanthoside, nyctanthic acid and β-sitosterol from the seeds [5–7]. Iridoids in general show a wide spectrum of biological activity and arbortristoside A showed 80% regression of methyl cholanthrene induced fibrosarcoma.

RESULTS AND DISCUSSION

Arbortristoside A (**1**), $C_{27}H_{34}O_{13}$, mp 226–228°, $(\alpha)_D$ −90° (MeOH; *c* 1.16) was identified as an iridoid glucoside on preliminary examination of its spectroscopic data and chemical reactions. It formed a pentaacetate, mp 88–90°. Enzyme hydrolysis of arbortristoside A with β-glucosidase afforded D-glucose and alkaline hydrolysis gave *p*-methoxy cinnamic acid. Its UV spectrum (λ_{max}^{EtOH} nm 235, 300 (sh) and 310) also suggested the presence of a *p*-methoxy cinnamoyl moiety and an α,β unsaturated ester in **1**. Mannich hydrolysis of arbortristoside A yielded the same aglucone as was obtained by enzyme hydrolysis. The ^{1}H NMR spectrum of the aglucone showed that it retained the *p*-methoxy cinnamoyl group as shown by the AB quartet at δ6.44 and 7.74 (AB *q*, J = 16 Hz). Hence the possibility of cinnamoyl group being present as an ester attached to the sugar moiety as is found in some iridoids (e.g. schrophulariosides, picroside and ladroside) was ruled out. The *p*-methoxy cinnamoyl group was placed at C-6 as the signal corresponding to this was observed at δ4.98 (*dd*, J = 4.5 and 1.5 Hz) in the ^{1}H NMR spectrum of the aglucone. As arbortristoside A formed a pentaacetate, it should have another hydroxyl group and this was placed at C-7, as H-7 appeared as a triplet at δ4.1 (J = 4.5 Hz). Carbon-8 was substituted by a methyl group which appeared as a doublet at δ1.07 (J = 6.5 Hz) in the pentaacetate (Table 1). The structure of arbortristoside A was further supported by ^{13}C measurements [8] (Table 2).

Treatment of arbortristoside A with alkali under mild conditions yielded *p*-methoxy cinnamic acid and another acid which was subsequently methylated with diazomethane. The resulting product was found to be identical in all respects with an authentic sample of 6β-hydroxy loganin, an iridoid glucoside isolated from the leaves of *Fouquieria columnaris* by Jensen and Nielsen [9]. The stereochemistry of the methyl group at C-8 is thus confirmed as β. Further, the C-9 shift (44.6 ppm) of arbortristoside A in the ^{13}C NMR spectrum was in good agreement with the theoretical value expected for a C-8 β methyl configuration (Damtoft *et al.* [10]).

Arbortristoside B (**2**) $C_{26}H_{32}O_{15}$, amorphous hygroscopic powder, $(\alpha)_D$ −69° (MeOH; *c* 1.66) formed an octaacetate, mp 156–158°, with acetic anhydride and sodium acetate and a dimethyl ether with diazomethane.



	R^1	R^2	R^3
1	Me	H	Me
2	CH_2OH	OH	H

Table 1. ^{1}H NMR data

H	Arbortristoside A (DMSO-d_6)	Arbortristoside A pentaacetate* ($CDCl_3$)	Aglucone of arbortristoside A ($CDCl_3$)	Arbortristoside B octaacetate† ($CDCl_3$)
1	5.35 *d*, J = 8 Hz	5.35 *m*	5.4	5.4 *d*, J = 8 Hz
3	7.42 *s*	7.47 *s*	7.5 *s*	7.39 *s*
5	—	3.1 *dd*, J = 7.5 & 1.5 Hz	3.15 *dd*, J = 7.5 & 1.5 Hz	3.05 *dd*, J = 7.5 & 1.5 Hz
6	4.95 *m*	5.35 *m*	4.98 *dd*, J = 4.5 & 1.5 Hz	5.4
7	4.1 *t*, J = 4.5 Hz	—	4.3	—
8	2.15 *m*	—	—	—
9	2.15 *m*	2.25 *m*	2.26 *m*	2.38 *m*
10	1.00 *s* (*br*)	1.07 *d*, J = 6.5 Hz	1.15 *d*, J = 6.5 Hz	4.25 *m*
12	3.65 *s*	3.7 *s*	3.75 *s*	3.7 *s*
α	6.47 AB *q*, J = 16 Hz	6.32 AB *q*, J = 16 Hz	6.44 AB *q*, J = 16 Hz	6.36 AB *q*, J = 16 Hz
β	7.5 AB *q*, J = 16 Hz	7.65 AB *q*, J = 16 Hz	7.74 AB *q*, J = 16 Hz	7.65 AB *q*, J = 16 Hz
Aromatic	6.95 (*d*, J = 10 Hz, H-3″ and H-5″)	6.93 (*d*, J = 10 Hz, H-3″ and H-5″)	6.95 (*d*, J = 10 Hz, H-3″ and H-5″)	7.21 (*d*, J = 10 Hz, H-5″
protons	7.65 (*d*, J = 10 Hz, H-2″ and H-6″)	7.33 (*d*, J = 10 Hz, H-2″ and H-6″)	7.55 (*d*, J = 10 Hz, H-2″ and H-6″)	7.39 (*d*, J = 2 Hz, H-2″)
	3.79 (*s*, –OMe)	3.85 (*s*, –OMe)	3.84 (*s*, –OMe)	7.45 (*dd*, J = 2Hz and 10 Hz, H-6″)
Sugar	4.95 (*m*, H-1′)	5.35 (*m*, H-1′)	—	5.4 (*d*, J = 8 Hz)
protons	4.48 (*d*, J = 9 Hz, –C$\underline{H}_2$OH)	4.28 (*m*, –C$\underline{H}_2$OAc),		4.25 (*m*, –C$\underline{H}_2$OAc)
		4.8–5.25 (*m*, >CHOAc of glucose)		4.7–5.3 (*m*, >CHOAc of glucose)

*Acetoxyl protons from five OAc groups at δ1.91, 2.00, 2.02 and 2.1.

†Acetoxyl protons from six OAc groups at δ1.88, 1.97, 2.02, 2.05 and those of phenolic acetates at 2.28.

Table 2. ^{13}C NMR data

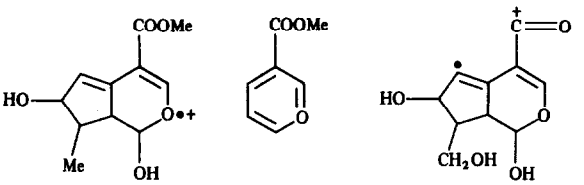
C	Arbortristoside A (DMSO-d_6)	Arbortristoside B octaacetate* ($CDCl_3$)
1	95.1	94.5
3	152.1	151.7
4	108.5	108.8
5	42	39.4
6	76.7	76.4
7	70	72
8	35	35.3
9	44.6	41.6
10	14.9	63.4
11	167.2	168
12	51.1	51.6
1′	98.6	96.02
2′	73	70.6
3′	77.2	72.4
4′	70	68.1
5′	75.7	72.4
6′	61.2	61.5
1″	126.7	133.0
2″	130	124.1
3″	114.4	142.6
4″	161.1	143.8
5″	114.4	123.0
6″	130	126.5
α	144.2	143.8
β	115.6	118.5
CO	166.2	166.2
–OMe	55.3	—

*Additional signals from acetoxy groups at 170.6, 170.2, 169.9, 169.6, 169.4, 169.1 ppm (C=O) and 20.6, 20.1 ppm (Me).

Enzyme hydrolysis of arbortristoside B with β-glucosidase gave D-glucose and an aglucone. Mannich hydrolysis of arbortristoside B yielded the same aglucone which still retained the caffeoyl group. The dimethyl ether on alkaline hydrolysis gave 3,4-dimethoxy cinnamic acid. Thus arbortristoside B had two more oxygen atoms than arbortristoside A. One extra oxygen was accommodated in the aromatic ring. The lack of a C-methyl group and the presence of an extra $-CH_2OH$ group (^1H NMR δ 4.25, ^{13}C NMR 63.4, *t*) showed the presence of a $-CH_2OH$ group at C-8 in arbortristoside B, instead of the methyl group found in arbortristoside A. Placement of the other functional groups and stereochemistry was deduced by analogy with arbortristoside A and by measurement of ^{13}C NMR shifts.

As expected, the mass spectrum of arbortristoside A had peaks at *m/z* 226 (fragment **a**), 178 (*p*-methoxy cinnamic acid), 161 (*p*-methoxy cinnamoyl), 139 (fragment **b**), arbortristoside B showed peaks at *m/z* 210 (fragment **c**), 180 (caffeic acid), 163 (caffeoyl) and 139 (fragment **b**) [11].

The structure proposed for arbortristoside B is therefore identical to 6-caffeoyl nyctanthoside. Removal of the 6-caffeoyl group chemically yielded nyctanthoside and therefore the stereochemistry of the various centres are the same as those reported for nyctanthoside which includes the latest assignment of the β configuration to the $-CH_2OH$ group on C-8 [9].



a *m/z* 226 **b** *m/z* 139 **c** *m/z* 210

EXPERIMENTAL

Mps: uncorr; ^1H NMR: 5–10% solns in DMSO-d_6 and $CDCl_3$ using TMS as int. standard; CC: silica gel (Acme's) 100–200 mesh. The separation was monitored by TLC (silica gel G).

Extraction and isolation. The seeds of *N. arbor-tristis* were collected from Jaipur, India. A specimen has been deposited at the herbarium of this Institute under the registry No. 701 and is available for inspection. Coarsely powdered seeds with the kernels (2 kg) were first defatted and then extracted with EtOH by cold percolation. The marc was then extracted with hot MeOH.

Isolation of arbortristoside A. The EtOH extract was evaporated to dryness *in vacuo*, treated with H_2O and extracted with Et_2O, EtOAC and *n*-BuOH. The *n*-BuOH extract was dried and triturated with Me_2CO. The solid which separated was washed with 2 N HCl and then with hot H_2O and was crystallised from $CHCl_3$–MeOH. The mother liquor was subjected to CC over silica gel (1 kg) and eluted with $CHCl_3$–MeOH (9:1) to yield more of compound **1** (2 g), mp 226–228°, $[\alpha]_D$ −90° (MeOH; *c* 1.16) (Found C, 57.45; H, 5.77. $C_{27}H_{34}O_{13}$ requires: C, 57.24; H, 6.01%). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1715, 1660, 1630, 1540, 1470, 1300, 1290, 1085, 1025, 950, 870, 840, 770; UV λ_{max} nm 235, 300 (sh) and 310; MS *m/z*: 226 [**a**]$^+$, 198 [**a** − H_2O]$^+$, 178 [*p*-methoxy cinnamic acid]$^+$, 161 [*p*-methoxy cinnamoyl moiety]$^+$, 139 [**b**]$^+$.

Acetylation of arbortristoside A. Arbortristoside A (**1**) (150 mg) was refluxed with Ac_2O (3 ml) and freshly fused NaOAc in an oil bath for 4 hr. Work-up in the usual way afforded the pentaacetate mp 85–87° (120 mg) (crystallized from dil. alcohol). (Found: C, 57.05; H, 5.84. $C_{37}H_{44}O_{18}$ requires: C, 57.22; H, 5.67%.)

Alkaline hydrolysis of arbortristoside A. Arbortristoside A (200 mg) was refluxed with methanolic KOH for 2 hr. After usual work-up, the acidic portion yielded *p*-methoxy cinnamic acid, mp 170° (crystallized from EtOH). The acid was found to be identical in all respects with a synthetic sample.

Enzyme hydrolysis of arbortristoside A. Arbortristoside A (150 mg) in MeOH (10 ml) was incubated with a soln of β-glucosidase (100 mg) in H_2O (10 ml) at 37° for 1 week. The aglucone was obtained in poor yield and the sugar was identified as D-glucose by PC.

Mannich hydrolysis of arbortristoside A. To a soln of arbortristoside A (250 mg) in Me_2CO (19 ml), conc. HCl (0.3 ml) was added and the mixture was kept at room temp. for about 20 days. The mixture was worked up and the aglucone was purified by preparative TLC.

Isolation of arbortristoside B. The *n*-BuOH soluble fraction from the hot MeOH extract was subjected to CC over silica gel. The $CHCl_3$–MeOH (17:3) eluates yielded arbortristoside B (**2**) (1 g), amorphous hygroscopic powder, $[\alpha]_D$ −69° (MeOH; *c* 1.66). IR ν_{max}^{KBr} cm^{-1}: 3350, 2920, 1690, 1630, 1520, 1440, 1270, 1080, 950, 860, 810, 770; MS *m/z*: 210 [**c**]$^+$, 180 [caffeic acid]$^+$, 163 [caffeoyl]$^+$ and 139 [**b**]$^+$.

Acetylation of arbortristoside B. The octaacetate was prepared in the same way as that of arbortristoside A, mp 156–158°

(crystallized from dil. alcohol). (Found: C, 54.6; H, 5.3 $C_{42}H_{48}O_{23}$ requires: C, 54.78; H, 5.22%.)

Methylation of arbortristoside B. Arbortristoside B (250 mg) in MeOH (10 ml) was treated with an ethereal soln of CH_2N_2 at 0°. After 3 days, the mixture was worked up in the usual way and the dimethyl ether was purified by CC over silica gel.

Alkaline hydrolysis of the dimethyl ether. The dimethyl ether was refluxed with methanolic KOH for 2 hr. After usual work-up, 3,4-dimethoxy cinnamic acid was obtained from the acidic fraction, mp 179–180° (crystallized from H_2O), and found to be identical in all respects with a synthetic sample.

Enzyme hydrolysis of arbortristoside B. Arbortristoside B was hydrolysed with β-glucosidase in the same way as arbortristoside A. The aglucone was obtained in poor yield and the sugar was identified as D-glucose by PC.

Mannich hydrolysis of arbortristoside B. Mannich hydrolysis was done as before. The yield of the aglucone was poor.

Conversion of arbortristoside A to 6β-hydroxyloganin. Arbortristoside A (250 mg) was treated with 0.5 N/NaOH (2.5 ml) and the mixture heated in water bath for 2 to 3 min. The soln was acidified with 1 N HCl, and then extracted with Et_2O and *n*-BuOH successively. The Et_2O extract yielded *p*-methoxy cinnamic acid.

The BuOH extract was evapd *in vacuo*, and was taken up in MeOH. The soln was then treated with an ethereal soln of CH_2N_2 at 0°. The mixture after usual work-up and purification through a silica gel column yielded crystalline material, mp 220–222°, which was found to be identical in all respects with an authentic sample of 6β-hydroxyloganin (mp, mmp and TLC).

Conversion of arbortristoside B to nyctanthoside. Arbortristoside B (250 mg) was hydrolysed with 0.5 N NaOH (2.5 ml) in the same way as arbortristoside A. The soln was acidified and extracted with *n*-BuOH. The BuOH extracted material was methylated with CH_2N_2. The methyl ester on purification through a column of silica gel yielded a gum which was found to be identical with an authentic sample of nyctanthoside (TLC and IR).

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REFERENCES

1. Bentham, G. and Hooker, J. D. (1862) *Genera Plantarum*, London.
2. Stant, M. Y. (1952) *Kew. Bull.* 273.
3. Airy Shaw, H. K. (1952) *Kew Bull.* 271
4. Das, V. S. R. and Rao, K. N. (1966) *Naturwissenschaften* **53**, 439.
5. Rimpler, H. and Jinghanns, J. U. (1975) *Tetrahedron Letters* **29**, 2423.
6. Turnbull, J. H., Vasistha, S. K., Wilson, W. and Woodger, R. (1957) *J. Chem. Soc.* 569.
7. Rimpler, H. (1978) *Planta Med.* **33**, 313.
8. Chaudhuri, R. K., Fatma, U., Yazar, A., Sticher, O. and Winkler, T. (1980) *Tetrahedron* **36**, 2317.
9. Jensen, S. R. and Nielsen, B. J. (1982) *Phytochemistry* **21**, 1623.
10. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1981) *Phytochemistry* **20**, 2717.
11. Bobbitt, J. M. and Segebarth, K. P. (1969) *Cyclopentanoid Terpene Derivatives*, pp. 112–113. Marcel Dekker, New York.