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Chemical Constituents from the Flowers of *Nyctanthes arbor-tristis*

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ABSTRACT Activity guided fractionation of an ethanol extract from the flowers of *Nyctanthes arbor-tristis* led to the isolation of an antiplasmodial cyclohexylethanoid, rengyolone (**1**); a new iridoid glucoside, 6-O-*trans*-cinnamoyl-7-O-acetyl-6b-hydroxyloganin (**2**); and three known iridoid glucosides, arborside C (**4**), 6b-hydroxyloganin (**6**) and nyctanthoside (**7**). Compound **1** and its acetate derivative (**1a**) exhibited antiplasmodial activity against *Plasmodium falciparum*. Chemical structures of **2** and its acetate (**2a**) were elucidated by spectral analyses.

KEYWORDS: *Nyctanthes arbor-tristis* Linn, Verbenaceae, antiplasmodial constituent, cyclohexylethanoid, iridoid glucosides.

INTRODUCTION

The leaves of *Nyctanthes arbor-tristis* Linn. are used extensively in Ayurvedic medicine for the treament of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic.[1,2] Earlier phytochemical studies on this plant resulted in the isolation of a number of iridoid glycosides from the leaves[3-7] and the seeds.[8-10] Three carotenoid glucosides were also isolated from the corolla tubes of the plant.[11] A new phenylpropanoid glycoside (nyctoside A) has been isolated from the seeds[12] and desrhamnosylverbascoside from the leaves.[13]

In continuation of our work on bioactive substances from Thai medicinal plants, a bioactivity-guided phytochemical investigation was carried out on the flowers of *N. arbor-tristis* and this led to the isolation of a cyclohexylethanoid, rengyolone (**1**) as an antimalarial principle. A new iridoid glucoside, 6-*O*-*trans*-cinnamoyl-7-*O*-acetyl-6β-hydroxyloganin (**2**) and three known iridoid glucosides, arborside C (**4**) ,[4] 6β-hydroxyloganin (**6**)[8,14] and nyctanthoside (**7**)[3] were also isolated from the same plant

MATERIALS AND METHODS

General

Melting points are uncorrected. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were measured with a Jasco A-302 spectrophotometer. ^{1}H- and ^{13}C-NMR were recorded on a Bruker Avance-400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometer. Low-resolution mass spectra were run on a Hewlett Packard 5989B spectrometer and high-resolution mass spectra on a Kratos Concept ISQ mass spectrometer.

Plant material

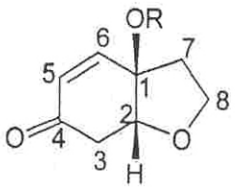
The flowers of *N. arbor-tristis* were collected from Sanamchan Palace Campus, Silpakorn University, Nakorn Pathom, Thailand, in 1998. The voucher specimen (BRU. 131) was deposited at the National Center for Genetic Engineering and Biotechnology (BIOTEC), 73/1 Rama 6 Rd., Rajdhevee, Bangkok 10400, Thailand.

Antiplasmodial assay

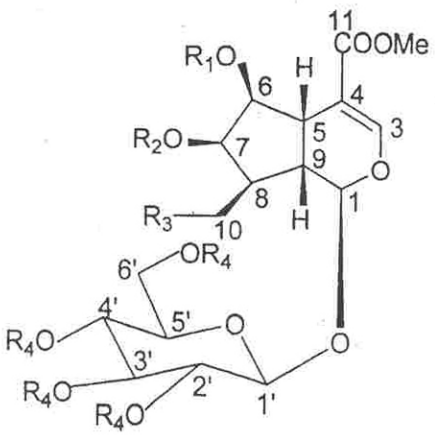
The *Plasmodium falciparum* (K1, multidrug resistant strain) was cultured continuously according to the method of Trager and Jensen.[21] The quantitative assessment of the antiplasmodial activity *in vitro* was performed by means of the microculture radioisotope technique based upon the method described by Desjardins *et al*.[22] The inhibitory concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 0.16 μg/ml (0.31 μM) was observed for the standard sample, chloroquine diphosphate, in the same test.

Chemical Constituents from the Flowers of

Nyctanthes arbor-tristis



1 : R = H
1a : R = Ac



	R_1	R_2	R_3	R_4
2:	*trans*-cinnamoyl	Ac	H	H
2a:	*trans*-cinnamoyl	Ac	H	Ac
3:	Ac	*trans*-cinnamoyl	H	H
3a	Ac	*trans*-cinnamoyl	H	Ac
4	H	benzoyl	H	H
4a	Ac	benzoyl	H	H
5	benzoyl	H	H	H
5a	benzoyl	Ac	H	H
6	H	H	H	H
6a	Ac	Ac	H	Ac
7	H	H	OH	H
7a	Ac	Ac	OAc	Ac

Extraction and Isolation

The dried flowers of *N. arbor-tristis* (187 g) were extracted with EtOH at room temperature. After concentration under reduced pressure, the extract (76 g) was chromatographed on a silica gel (70-230 mesh, 1.5 kg) column (column 1) eluted with a gradient mixture of hexane-EtOAc, EtOAc, EtOAc-MeOH and EtOAc-MeOH-H_2O to give 17 fractions. Fractions 3 (0.8 g) and 4 (1.3 g) (eluted with hexane-EtOAc, 1:1) were combined and purified over a silica (70-230 mesh, 200 g) column gradiently eluted with CH_2Cl_2-MeOH and CH_2Cl_2-MeOH-H_2O to give compound **1** (eluted with CH_2Cl_2-MeOH-H_2O, 50:3:1) as a colorless oil (496 mg). Fraction 5 (6.6 g) (eluted with hexane-EtOAc, 1:9 in column 1) was chromatographed over a silica gel (70-230 mesh, 350 g) column eluted with CH_2Cl_2-MeOH-H_2O gradient to give crude 6-O-*trans*-cinnamoyl-7-*O*-acetyl-6β-hydroxyloganin (**2**) (eluted with CH_2Cl_2-MeOH-H_2O, 30:3:1) as a colorless oil (400 mg) and crude arborside C (**4**) (eluted with CH_2Cl_2-MeOH-H_2O, 20:3:1) as a light yellow oil (495 mg). Fractions 6 (3.0 g) and 7 (1.5 g) (eluted with EtOAc and EtOAc-MeOH, 50:1)were combined and chromatographed on a silica gel (70-230 mesh, 225 g) column gradiently eluted with CH_2Cl_2-MeOH-H_2O to give crude 6β-hydroxyloganin (**6**) (eluted with CH_2Cl_2-MeOH-H_2O, 30:3:1) as a light yellow oil (234 mg) and crude nyctanthoside (**7**) (eluted with CH_2Cl_2-MeOH-H_2O, 10:3:1) as a yellow oil (541 mg).

*Compound **1*** was obtained as a colorless oil; HRMS *m/z*: $[M]^+$ 154.0630. Calc. for $C_8H_{10}O_3$ 154.0629; $[\alpha]_D^{26}$ +0.28° (*c*, 3.24, MeOH) [lit. (15) +0.26°]; UV (MeOH) λ_{max} (log ε) nm : 212.5(3.85); IR ν_{max}^{neat} cm^{-1} : 3400 (broad), 1670 (broad), 1390, 1270, 1200, 1140, 1110, 1090; ^{1}H-NMR ($CDCl_3$) : δ 6.76 (1H, dd, J = 1.5, 10.2 Hz, H-6), 6.01 (1H, d, J= 10.2 Hz, H-5), 4.24 (1H, ddd, J = 1.5, 4.8, 5.8 Hz, H-2), 4.07 (1H, ddd, J = 6.5, 8.1, 8.7 Hz, H_a-8), 3.95 (1H, ddd, J = 6.3, 8.4, 8.7 Hz, H_b-8), 2.78 (1H, dd, J = 4.8, 16.9 Hz, H_a-3), 2.61 (1H, ddd, J= 0.5, 5.8, 16.9 Hz, H_b-3), 2.33 (1H, ddd, J = 6.3, 8.4, 13.0 Hz, H_a-7), 2.22 (1H, br ddd, J = 6.5, 8.1, 13.0 Hz, H_b-7); ^{13}C-NMR ($CDCl_3$) : δ 197.1 (C-4), 148.4 (C-6), 128.4 (C-5), 81.4 (C-2), 75.3 (C-1), 66.2 (C-8), 40.0 (C-3), 39.5 (C-7); EIMS *m/z* (rel. int.) : 154$[M]^+$(8), 137(15), 131(24), 112(46), 110(74), 82(100), 70(74), 55(92), 43(86).

*Acetylation of compound **1***. A mixture of compound **1** (110 mg), pyridine (1.0 ml) and acetic anhydride (1.0 ml) was heated at 85°C for 2h. After the workup, the crude acetate derivative (**1a**) was purified by preparative TLC [GF_{254}, hexane:EtOAc (3:1)] to give 1a as a colorless oil (68.9 mg); $[\alpha]_D^{26}$ +0.28° (*c*, 2.47, MeOH); HRMS *m/z*: $[M]^+$ 196.0739. Calc. for $C_{10}H_{12}O_4$ 196.0735; UV (MeOH) λ_{max}(log ε) nm : 215.6(3.94); IR ν_{max}^{neat} cm^{-1} : 1740, 1688, 1373, 1241, 1073, 1023; ^{1}H-NMR ($CDCl_3$) : δ 6.97 (1H, br dd, J = 1.8, 10.2 Hz, H-6),6.06 (1H, br d, J = 10.2 Hz, H-5), 4.36 (1H, ddd, J = 1.8, 3.6, 4.9 Hz, H-2), 3.94 (2H, second order m, H_aH_b-8), 2.94 (1H, dd, J = 5.0, 17.2 Hz, H_a-3), 2.71 (1H, br dd, J = 3.6, 17.2 Hz, H_b-3), 2.48 (2H, second order m, H_aH_b-7), 2.09 (3H, s, OAc); ^{13}C-NMR ($CDCl_3$) : δ 195.8 (C-4), 170.1 (O<u>CO</u>CH_3), 144.3 (C-6), 129.4 (C-5), 80.8 (C-1), 79.8 (C-2), 65.8 (C-8), 39.7 (C-3), 37.8 (C-7), 21.3 (OCO<u>C</u>H_3); EIMS *m/z* (rel. int.): 197 $[M+H]^+$(100), 137(75).

*6-O-trans-Cinnamoyl-7-O-acetyl-6β-hydroxyloganin (**2**)* (369 mg) was further purified on a column of silica gel (70-230 mesh, 35 g) eluted with EtOAc and EtOAc-MeOH (100:1 and 50:1) to give **2** (impure) as a colorless solid (245 mg) and **2** containing a trace of the isomeric 6-O-acetyl-7-O-cinnamoyl-6β-hydroxyloganin (**3**) as a colorless solid (64.1 mg). mp 93-96°C ; $[\alpha]_D^{30}$ –104.6° (*c* 1.45, CH_2Cl_2); HRFABMS *m/z*: $[M+H]^+$ 579.2057. Calc. for $C_{28}H_{35}O_{13}$ 579.2075; UV (MeOH) λ_{max}(log ε) nm : 222.8(4.29), 233sh (4.15), 277.6(4.28); IR ν_{max}^{Nujol} cm^{-1}: 3425, 1712, 1635, 1285, 1242, 1168, 1076; FABMS *m/z* (rel. int.): 601$[M+Na]^+$(100), 579$[M+H]^+$(58), 417(48), 385(50), 293(20), 270(62); ^{1}H-and ^{13}C-NMR data see Table 1.

*Acetylation of Compound **2***. Compound **2** (51 mg) was acetylated by the same manner for **1** to give the acetate derivative **2a** as a colorless solid (47 mg); mp 80-82°C; $[\alpha]_D^{30}$ -95.4° (*c* 1.40, CH_2Cl_2); HRFABMS *m/z*: $[M+H]^+$ 747.2478. Calc. for $C_{36}H_{43}O_{17}$ 747.2497; UV (MeOH) λ_{max} (log ε) nm : 223(4.29), 232sh(4.12), 278(4.30); IR ν_{max}^{Nujol} cm^{-1}: 1749, 1715, 1637, 1371, 1225, 1071, 1043; FABMS *m/z* (rel. int.): 747$[M+H]^+$(15), 331(100), 271(12); ^{1}H- and ^{13}C-NMR data see Table 2.

*6-O-Acetyl-7-O-trans-cinnamoyl-6β-hydroxyloganin pentaacetate (**3a**).* ^{1}H-NMR ($CDCl_3$): δ 0.91 (d, J = 6.8 Hz, H-10), 2.10 (br sextet, H-8), 2.29 (ddd, J = 2.4, 9.0, 10.2 Hz, H-9), 2.98 (ddd, J = 1.2, 4.0, 9.0 Hz, H-5), 3.58 (s, OCH_3), 4.34 (dd, J = 4.2, 12.6 Hz, Hb-6'), 4.83 (d, J = 8.0 Hz, H-1'), 5.02 (dd, J = 8.0, 9.5 Hz, H-2'), 5.113 (t, J = 9.5 Hz, H-4'), 5.21 (dd, J = 4.0, 5.6 Hz, H-7), 5.21 (partly overlapped signal, H-1), 5.225 (t, J = 9.5 Hz, H-3'), 5.34 (t, J = 4.0 Hz, H-6), 6.35 (d, J = 16.0 Hz, Hα), 7.34 (br s, H-3), 7.60 (d, J = 16.0 Hz, Hβ).

*Arborside C (**4**)* (130 mg) was further purified on a column of silica gel (70-230 mesh, 10 g) eluted with EtOAc and EtOAc-MeOH (50:1 and 25:1) to give pure **4** as colorless needles (42 mg); mp 210-212°C [lit. (4) 210-212°C]. ^{1}H- and ^{13}C-NMR data see Table 3.

*Isoarborside C (**5**).* ^{1}H-NMR (DMSO-d_6/D_2O): δ 1.08 (d, J = 7.0 Hz, H-10), 1.95 (m, H-8), 2.18 (m, H-9), 3.18 (ddd, J = 1.5, 5.0, 9.0 Hz, H-5), 3.47 (s, OCH_3), 4.07 (t, J = 4.2 Hz, H-7), 4.49 (d, J = 7.0 Hz, H-1'), 5.03 (dd, J = 4.2, 5.0 Hz, H-6), 5.36 (d, J = 4.4 Hz, H-1), 7.42 (d, J = 1.3 Hz, H-3), 7.44 (t, J = 8.4 Hz, H-3" and H-5"), 7.65 (tt, J = 1.4, 8.4 Hz, H-4"), 7.99 (dd, J = 1.4, 8.4 Hz, H-2" and H-6"). ^{13}C-NMR (DMSO-d_6): δ 14.0 (C-10), 35.6

Table 1. ^{1}H-NMR and ^{13}C-NMR spectral data of compound **2** in CD_3OD.

Position	δH	COSY	δC	HMBC
1	5.46 (d, 4.2)	H-9	96.7	H-3, H-5, H-8, H-9, H-1′
3	7.48 (d, 1.3)	H-5	153.8	H-1, H-5
4	-	-	109.8	H-3, H-5, H-6, H-9
5	3.27 (ddd, 1.3, 4.2, 8.8)	H-3, H-6, H-9	37.2	H-1, H-6, H-7, H-9, H-10
6	5.305 (m)	H-5, H-7	79.2	H-5, H-9
7	5.310 (m)	H-6, H-8	76.0	H-5, H-9, H-10, OAc
8	2.27 (br sextet, 8.0)	H-7, H-9, H-10	37.1	H-1, H-5, H-6, H-7, H-10
9	2.35 (dt, 4.2, 8.8)	H-1, H-5, H-8	45.7	H-1, H-5, H-6, H-7, H-10
10	1.11 (d, 6.8)	H-8	14.1	H-7, H-9
11	-	-	168.8	H-3, OCH_3
OCH_3	3.65 (s)	-	51.9	-
1′	4.67 (d, 8.0)	H-2′	100.0	H-1, H-2′, H-3′
2′	3.22 (dd, 8.0, 9.1)	H-1′, H-3′	74.6	H-1′, H-3′, H-4′
3′	3.40 (t, 9.1)	H-2′, H-4′	77.8	H-1′, H-2′, H-4′
4′	3.29 (t, 9.1)	H-3′, H-5′	71.5	H-3′, Hab-6′
5′	3.34 (overlapped signal)	H-4′, Hab-6′	78.4	Ha-6′, H-3′, H-4′
6a′	3.68 (dd, 6.4, 12.0)	H-5′, Hb-6′	62.7	H-4′, H-5′
6b′	3.93 (dd, 2.0, 12.0)	H-5′, Ha-6′		
CO(cinnamoyl)	-	-	167.4	H-6, Hα, Hβ
α	6.51 (d, 16.0)	Hβ	118.5	Hβ
β	7.70 (d, 16.0)	Hα	146.6	Hα, H-2", H-6"
1"	-	-	135.5	Hα, Hβ, H-2", H-3", H-5", H-6"
3",4",5"	7.42 (m)	H-2", H-6" (3",5")	130.0	H-2", H-6"
		(4")	131.6	H-2", H-6"
2",6"	7.62 (m)	H-3", H-4", H-5"	129.2	Hβ, H-4"
$\underline{CH_3}CO$	2.07 (s)	-	20.7	-
$CH_3\underline{C}O$	-	-	172.1	$\underline{CH_3}CO$, H-7

Table 2. ^{1}H-NMR and ^{13}C-NMR spectral data of compound **2a** in $CDCl_3$.

Position	δH	COSY	δC	HMBC
1	5.322 (d, 2.4)	H-9	94.2	H-5, H-8, H-9, H-1′
3	7.385 (d, 1.4)	H-5	150.8	H-1, H-5
4	-	-	110.1	H-3, H-5, H-6, H-9
5	3.09 (ddd, 1.4, 3.6, 9.0)	H-3, H-6, H-9	35.5	H-1, H-6, H-7, H-9
6	5.355 (br t, 3.6, 4.4)	H-5, H-7	77.2	H-5, H-9
7	5.320 (dd, 4.4, 5.8)	H-6, H-8	74.6	H-5, H-6, H-10, OAc(δ 2.05)
8	2.14 (br sextet,)	H-7, H-9, H-10	35.9	H-1, H-5, H-6, H-9, H-10
9	2.56 (ddd, 2.4, 9.0, 10.2)	H-1, H-5, H-8	44.8	H-1, H-5, H-6, H-7, H-8, H-10
10	1.07 (d, 6.8)	H-8	13.4	H-7, H-8, H-9
11	-	-	166.5	H-3, OCH_3
OCH_3	3.66 (s)	-	51.5	-
1′	4.86 (d, 8.0)	H-2′	95.9	H-1, H-2′, H-3′
2′	4.99 (dd, 8.0, 9.5)	H-1′, H-3′	70.5	H-3′, H-4′, OAc (δ 1.91)
3′	5.23 (t, 9.5)	H-2′, H-4′	72.4	H-1′, H-2′, H-4′, OAc (δ 2.01)
4′	5.11 (t, 9.5)	H-3′, H-5′	68.2	H-3′, H-5′, Hab-6′, OAc (δ 2.04)
5′	3.76 (ddd, 2.1, 4.2, 9.5)	H-4′, Hab-6′	72.3	H-1′, H-4′, Hab-6′
6a′	4.16 (dd, 2.1, 12.6)	H-5′, Hb-6′	61.7	H-4′, OAc (δ 2.11)
6b′	4.322 (dd, 4.2, 12.6)	H-5′, Ha-6′		H-4′
CO(cinnamoyl)		-	165.5	H-6, Hα, Hβ
α	6.44 (d, 16.0)	Hβ	117.6	Hβ
β	7.71 (d, 16.0)	Hα	145.3	Hα, H-2", H-6"
1"	-	-	134.3	Hα, Hβ, H-2", H-3",H-5", H-6"
2", 6"	7.55 (m)	H-3", H-4", H-5"	128.2	$H_{b'}$ H-4"
3", 4", 5"	7.40 (m)	H-2", H-6"(3", 5")	128.9	H-2", H-6"
		(4")	130.37	H-2", H-6"
5x$\underline{CH_3}$CO	1.91, 2.01, 2.04, 2.05, 2.11 (all s)		20.1, 20.6 (3x), 20.7	-
$CH_3\underline{CO}$	-	-	169.1, 169.4, 170.1(2x), 170.6	-

Table 3. ^{1}H-NMR and ^{13}C-NMR spectral data of arborside C (**4**) in DMSO-d_6/D_2O.

Position	δH	COSY	δC	HMBC
1	5.39 (d, 3.8)	H-9	95.6	H-3, H-5, H-8, H-9, H-1′
3	7.46 (d, 1.3)	H-5	152.3	H-1, H-5
4	-	-	108.9	H-3, H-5, H-6, H-9
5	2.93 (ddd, 1.3, 4.2, 8.2)	H-3, H-6, H-9	38.2	H-1, H-3, H-6, H-7, H-9
6	4.18 (t, 4.2)	H-5, H-7	76.3	H-5, H-9, H-8
7	5.12 (dd, 4.2, 7.0)	H-6, H-8	77.0	H-5, H-6, H-8, H-10
8	2.17 (m)	H-7, H-9, H-10	35.6	H-5, H-6, H-9, H-10
9	2.26 (m)	H-1, H-5, H-8	44.8	H-5, H-6, H-7, H-8, H-10
10	1.05 (d, 7.0)	H-8	15.2	H-7, H-8, H-9
11	-		167.9	H-3, H-5, OCH_3
OCH_3	3.65 (s)	-	51.8	-
1′	4.52 (d, 7.0)	H-2′	98.9	H-1, H-2′, H-3′
2′	3.01 (dd, 7.0, 8.0)	H-1′, H-3′	73.3	H-1′, H-3′
3′	3.21 (t, 8.0)	H-2′, H-4′	76.8	H-2′, H-4′
4′	3.075 (t, 8.0)	H-3′, H-5′	70.3	H-3′, H-5′
5′	3.20 (overlapped signal)	H-4′, Hab-6′	77.5	H-4′, Hab-6′
6a′	3.47 (dd, 5.0, 10.0)	Hb-6′, H-5′	61.4	H-5′
6b′	3.72 (dd, 1.0, 10.0)	Ha-6′, H-5′		
1"	-	-	130.3	H-3", H-5"
2", 6"	8.04 (dd, 1.4, 8.4)	H-3", H-4" H-5"	129.8	H-3", H-4", H-5"
4"	7.66 (tt, 1.4, 8.4)	H-2",H-3", H-5", H-6"	133.7	H-2", H-6"
3", 5"	7.54 (t, 8.4)	H-2′, H-4", H-6"	129.1	H-2", H-4", H-6"
CO (benzoyl)	-	-	166.1	H-7, H-2", H-6"
OH*	4.56 (t, 5.3), 4.95 (d, 5.3), 4.97 (d, 5.3), 5.01 (d, 5.3), 5.07 (d, 4.6)			

*Prior to addition of D_2O (1 drop) OH signals were observed at δ and signals of associated carbinyl protons showed one extra coupling.

(C-5), 37.2 (C-8), 43.8 (C-9), 51.5 (OCH_3), 71.9 (C-7), 81.0 (C-6), 95.6 (C-1), 98.8 (C-1'), 109.5 (C-4), 129.0(C-3", C-5"), 129.4 (C-2", C-6"), 133.6 (C-4"), 152.3 (C-3), 165.9 (CO benzoyl), 167.1 (C-11).

*Isoarborside pentaacetate(**5a**).* ^{1}H-NMR ($CDCl_3/C_6D_6$, 7:1): δ 1.04 (d, J = 6.9 Hz, H-10), 2.13 (m, H-8), 2.54 (ddd, J = 2.4, 9.6, 11.5 Hz, H-9), 3.18 (ddd, J = 1.2, 3.6, 9.6 Hz, H-5), 3.55 (s, OCH_3), 3.55 (overlapped, H-5'), 4.11 (dd, J = 2.4, 12.6 Hz, H-6a'), 4.28 (dd, J = 5.4, 12.6 Hz, H-6b'), 4.80 (d, J = 8.4 Hz, H-1'), 5.01 (dd, J = 8.4, 9.6 Hz, H-2'), 5.10 (t, J = 9.6 Hz, H-4'), 5.23 (t, J = 9.6 Hz, H-3'), 5.29 (d, J = 2.4 Hz, H-1), 5.35 (t, J = 4.4 Hz, H-7), 5.50 (t, J = 4.4 Hz, H-6), 7.32 (d, J = 1.2 Hz, H-3), 7.39 (t, J = 8.4 Hz, H-3" and H-5"), 7.49 (t, J = 8.4 Hz, H-4"), 8.04 (dd, J = 1.4, 8.4 Hz, H-2" and H-6"), 1.88, 1.91, 1.95, 1.97, 2.04 (all s, 5xOAc). ^{13}C-NMR ($CDCl_3$): δ 13.4 (C-10), 35.7 (C-5), 36.2 (C-8), 44.9 (C-9), 51.3 (OCH_3), 61.6 (C-6'), 68.1 (C-4'), 70.6 (C-2'), 72.2 (C-3'), 72.4 (C-5'), 74.5 (C-7), 77.6 (C-6), 94.4 (C-1), 96.0 (C-1'), 110.2 (C-4), 129.6 (C-1"), 129.7 (C-2", C-6"), 128.4 (C-3", C-5"), 133.0 (C-4"), 150.9 (C-3), 165.2 (CO benzoyl), 166.4 (C-11), 169.0, 169.3, 169.6, 170.0, 170.4 (5xAc), 20.0, 20.4 (2x), 20.6 (2x) (5xAc).

*6β-Hydroxyloganin (**6**)* (182 mg) was further purified by preparative TLC [GF_{254}, CH_2Cl_2-MeOH-H_2O (10:3:1), 4 runs] to give **6** as colorless needles (126 mg); mp 219-220°C [lit. (14) 220-222°C]. ^{1}H- and ^{13}C-NMR data see Tables 5 and 6.

*Nyctanthoside (**7**)* (287 mg) was rechromatographed on a silica gel (70-230 mesh, 25 g) column gradiently eluted with CH_2Cl_2-MeOH-H_2O to give **7** (eluted with CH_2Cl_2-MeOH-H_2O, 15:3:1) as a colorless resin (96 mg); $[\alpha]_D^{25}$ –66.4° (*c* 0.14, MeOH) [lit.(3) –65.1°]; UV (MeOH) λ_{max} (log ε) nm : 236.8 (3.94); IR ν_{max}^{Nujol} cm^{-1}: 3386, 1685, 1635, 1302, 1079. ^{1}H- and ^{13}C-NMR see Tables 5 and 6.

*Acetylation of compounds **4**, **6** and **7**.* Compounds **4** (52 mg), **6** (52 mg) and **7** (85 mg) were acetylated to give the acetate derivatives **4a** as a colorless solid (36 mg), crystallized from MeOH-H_2O as colorless solid, mp 158-159°C [lit.(4) 145-146°C] ^{1}H- and ^{13}C-NMR data see Table 4, **6a** as a colorless solid (40 mg), crystallized from MeOH as colorless needles, mp 123-125°C [lit. (14) 130-131.5°C] ^{1}H- and ^{13}C-NMR data see Tables 5 and **6** and **7a** as a colorless powder (47 mg), mp 179-181°C; $[\alpha]_D^{25}$ –62.4° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) nm : 230.2(4.01); IR ν_{max}^{Nujol} cm^{-1}: 1745, 1712, 1638, 1230, 1088. ^{1}H- and ^{13}C-NMR see Tables 5 and 6, respectively.

Results and Discussion

Compound **1**, obtained as a colorless oil, was identified as rengyolone by interpretation of the ^{1}H- and ^{13}C-NMR, COSY, HMQC and HMBC spectra. In particular the COSY spectrum showed long range cross peaks between H-2 and H-6, between HaHb-3 and H-5, and between Hb-3 and H-7. Full data for **1** and the acetate 1a are provided in the experimental. Rengyolone was first isolated from *Forsythia suspensa* (Oleaceae), an important plant of the crude drug "rengyo" (Forsythia Fructus).[15] It was also isolated, as a halleridone from the African medicinal plant *Halleria lucida* (Scrophulariaceae),[16] and as a cytotoxic constituent from *Cornus controversa* (Cornaceae).[17] It has since been isolated from the flowers of the Thai medicinal plant, *Millingtonia hortensis* (Bignoniaceae),[18] but has not previously been isolated from *Nyctanthes*. It has been suggested that rengyolone (halleridone) could arise biogenetically from a *p*-hydroxyphenylethanol precursor.[18-20]

Compound **2** was obtained as a colorless solid and its molecular formula was determined to be $C_{28}H_{34}O_{13}$ by HRFABMS and ^{13}C-NMR data (Table 1). The IR spectrum of **2** indicated the presence of hydroxyl group (3425 cm^{-1}), α, β-unsaturated ester carbonyl (1712 cm^{-1}) and enol-ether system (1635 cm^{-1}). ^{1}H- and ^{13}C-NMR spectral data of 2 (Table 1) were quite similar to those of 6β-hydroxyloganin (**6**) with the additional signals from *trans*-cinnamoyl and acetyl groups. A **2** proton muliplet (2nd order) centred at d 5.31 due to H-6 and H-7 in the ^{1}H-NMR spectrum of **2** suggested that the two acyl units, *trans*-cinnamoyl and acetyl, were located variously at C-6 and C-7 in **2**.

In view of the previous confusion in the literature concerning the location of ester functions in esters of 6β-hydroxyloganin (**10**) (arbortristoside A and B have been shown to have *p*-methoxycinnamoyl and caffeoyl ester groups respectively at C-7, and not at C-6 as originally proposed), care was needed to precisely locate the two acyl groups. Firstly, the H-5 resonance, obscured by glucose proton resonances, was located by gradient enhanced 1D TOCSY experiments using selective shaped pulses on the H-10 and H-8 resonances. Decoupling experiments involving H-5 and H-8 then confirmed that the upfield and downfield portions of the multiplet were due to H-6 and H-7, respectively (approx. δ values 5.305 and 5.310). The HMQC spectrum showed ^{13}C correlations at δ 79.2 and 76.0 with H-6/H-7. The low digitization in the F2 dimension did not allow a distinction. However, in the HMBC spectrum only the upfield ^{13}C resonance (δ 76.0) showed a long range connection with H-10 and therefore must be due to C-7. Moreover the signal showed also a correlation with the acetate methyl, confirming the location of the acetate group at C-7(this type of ^{4}J correlation, although of lower intensity, can be routinely observed). The acetate carbonyl (δC 172.1) showed correlations with H-7 and acetate methyl, while the cinnamoyl carbonyl (δC 167.4) was correlated with

Table 4. ^{1}H-NMR and ^{13}C-NMR spectral data of arborside C pentaacetate (**4a**) in $CDCl_3$ or $CDCl_3/C_6D_6$ (7:1).

Position	δH	COSY	δC	HMBC
1	5.32 (d, 2.4)	H-9	94.1	H-3, H-5, H-8, H-9, H-1′
3	7.38 (d, 1.2)	H-5	150.6	H-1, H-5
4	-	-	110.3	H-3, H-5, H-6, H-9
5	3.09 (ddd, 1.2, 4.2, 9.1)	H-3, H-6, H-9	35.5	H-3, H-6, H-7, H-9
6	5.34 (t, 4.2)	H-5, H-7	77.2	H-5, H-7, H-8, OAc (δ 1.92)
7	5.45 (dd, 4.2, 6.0)	H-6, H-8	75.4	H-5, H-6, H-10
8	2.153(m)	H-7, H-9, H-10	36.2	H-1, H-5, H-6, H-7, H-9, H-10
9	2.60 (ddd, 2.4, 9.1, 11.5)	H-1, H-5, H-8	44.9	H-5, H-6, H-7, H-8, H-10
10	1.06 (d, 6.9)	H-8	13.2	H-7, H-8, H-9
11	-	-	166.4	H-1, H-5, OCH_3
OCH_3	3.64 (s)	-	51.3	-
1′	4.81 (d, 8.4)	H-2′	95.9	H-1, H-2′, H-3′
2′	5.02 (dd, 8.4, 9.6)	H-1′, H-3′	70.6	H-1′, H-3′, H-4′, OAc (δ 1.89)
3′	5.23 (t, 9.6)	H-2′, H-4′	72.2	H-1′, H-2′, H-4′, H-5′,
4′	5.11 (t, 9.6)	H-3′, H-5′	68.1	OAc (δ 1.95) H-3′, H-5′, Hab-6′,
				OAc (δ 1.97)
5′	3.65 (ddd, 2.4, 5.4, 9.6)	H-4′, Hab-6′	72.4	H-1′, H-3′, H-4′, Hab-6′
6′a	4.10 (dd, 2.4, 12.6)	H-5′, Hb-6′	61.6	H-4′, H-5′, OAc (δ 2.03)
6′b	4.29 (dd, 5.4, 12.6)	H-5′, Ha-6′		
1"	-	-	129.6	H-2′,H-3", H-5", H-6"
2", 6"	8.01 (dd, 1.4, 8.4)	H-3", H-4", H-5"	129.6	H-3", H-4", H-5"
3", 5"	7.39 (t, 8.4)	H-2", H-6", H-4"	128.4	H-2", H-4", H-6"
4"	7.51 (tt, 1.4, 8.4)	H-2", H-3", H-5",	133.1	H-2", H-3", H-5", H-6"
		H-6"		
CO (benzoyl)			165.7	H-7, H-2", H-6"
5x$\underline{CH_3}$CO	1.89, 1.92, 1.95, 1.97,	-	20.0, 20.4(2x),	-
	2.03 (all s)		20.6(2x)	
5x$CH_3\underline{C}O$	-	-	169.0, 169.3,	-
			169.6, 170.0, 170.4	

Table 5. ^{1}H-NMR spectral data of **6** (CD_3OD), **6a** ($CDCl_3$), **7** (CD_3OD) and **7a** ($CDCl_3$).

Position	6	6a	7	7a
1	5.27 (d, 4.4)	5.28 (d, 2.6)	5.26 (d, 5.6)	5.38 (d, 2.7)
2	7.47 (d, 1.3)	7.35 (d, 1.3)	7.52 (d, 1.5)	7.41 (d, 1.4)
5	2.92 (ddd, 1.3, 6.2, 9.0)	2.98 (ddd, 1.4, 3.6, 9.0)	2.99 (ddd, 1.5, 6.5, 9.0)	3.02 (ddd, 1.4, 3.1, 8.4)
6	3.83 (dd, 4.0, 6.2)	5.24 (t, 4.3)	3.88 (dd, 4.0, 6.5)	5.45 (dd, 3.1, 4.0)
7	3.89 (dd, 4.0, 4.8)	5.17 (dd, 4.3, 5.4)	4.12 (dd, 4.0, 6.4)	5.30 (dd, 4.0, 7.8)
8	1.92 (ddq, 4.8, 7.9)	2.08 (m)	2.12 (br quintet, 7.0)	2.46 (quintet, 8.0)
9	2.16 (ddd, 4.4, 7.9, 9.0)	2.49 (ddd, 2.6, 9.0, 10.0)	2.29 (ddd, 5.6, 7.0, 9.0)	2.65 (dt, 2.7, 8.4)
10	1.02 (d, 7.9)	1.04 (d, 7.0)	-	-
10a		-	3.77 (dd, 5.1, 10.5)	4.12 (dd, 8.0, 12.4)
10b		-	3.81 (dd, 6.7, 10.5)	4.16 (dd, 8.0, 12.4)
OCH_3	3.73 (s)	3.67 (s)	3.75 (s)	3.71 (s)
1′	4.63 (d, 7.8)	4.83 (d, 8.4)	4.63 (d, 8.0)	4.83 (d, 8.4)
2′	3.18 (dd, 7.8, 9.0)	4.97 (dd, 8.4, 9.5)	3.20 (dd, 8.0, 9.0)	4.97 (dd, 8.4, 9.8)
3′	3.36 (t, 9.0)	5.22 (t, 9.5)	3.37 (t, 9.0)	5.22 (t, 9.8)
4′	3.28 (t, 9.0)	5.10 (t, 9.5)	3.30 (m)	5.09 (t, 9.8)
5′	3.30 (m)	3.74 (ddd, 2.4, 4.6, 12.4)	3.30 (m)	3.74 (ddd, 2.5, 4.5, 9.8)
6a′	3.65 (dd, 5.4, 11.8)	4.16 (dd, 2.4, 12.4)	3.66 (dd, 5.6, 12.0)	4.17 (dd, 3.1, 12.5)
6b	3.89 (dd, 1.9, 11.8)	4.31 (dd, 4.8, 12.4)	3.88 (dd, 1.9, 12.0)	4.26 (dd, 4.5, 12.5)
$\underline{CH_3}CO$	-	1.90, 2.00, 2.03, 2.04	-	1.90, 2.00, 2.04 (3x)
	-	2.07, 2.09 (all s)	-	2.07, 2.09 (all s)

Hα, Hβ and H-6. The assignment of the protons of the glucose moiety was made by 1D TOCSY experiments with a selective shaped pulse on H-1′. Further support came from COSY, HMQC and HMBC experiments (Table 1).

The ^{1}H-NMR spectrum ($CDCl_3$) of the pentaacetate (**2a**) was much clearer, with well-resolved signals, except that the resonances of H-7 (δ 5.320, dd, J = 4.4, 5.8 Hz) and H-1 (δ 5.322, d, J = 2.4 Hz) overlapped. Addition of deuterobenzene separated the signals. The ^{1}H- and ^{13}C-NMR spectra were fully assigned by decoupling, DEPT, COSY, HMQC and HMBC experiments. Again, C-7 (δ 74.6) showed correlations with H-10 and one acetate methyl group (δ 2.05), in addition to H-5 and H-6. The relative and absolute configurations of **2** were assigned by analogy with known iridoid glucosides.

The ^{1}H-NMR spectrum of **2** showed some weak signals in the baseline. In the spectrum of the acetate (**2a**) minor peaks were more clearly resolved. Close examination of the COSY spectrum at lower contour levels indicated some new connectivities. The complete subspectrum of an iridoid nucleus was revealed by 1D TOCSY experiments on the H-9 and H-10 signals. It would appear that the impurity was the isomeric 6-*O*-acetyl-7-*O*-*trans*-cinnamoyl-6β-hydroxyloganin (**3/3a**). ^{1}H-NMR assignments of **3a** are in the Experimental.

Compound **4** was identified as arborside C by interpretation of the 1D and 2D- NMR spectra. Further support came from analysis of the acetate (**4a**). It was found that after several months the ^{1}H- and ^{13}C-NMR spectra of arborside C had changed slightly. A new set of discrete signals had appeared, to the extent of about

Table 6. ^{13}C-NMR spectral data of **6** (CD_3OD), **6a** ($CDCl_3$), **7** (CD_3OD), **7a** ($CDCl_3$).

Position	6	6a	7	7a
1	97.9	94.2	98.8	94.4
3	153.7	150.8	154.4	151.7
4	111.4	110.1	110.3	108.5
5	39.6	35.3	39.1	35.1
6	80.5	77.0	79.8	76.3
7	75.3	74.7	73.6	71.7
8	38.8	35.7	45.6	39.0
9	45.6	44.7	40.7	42.0
10	14.0	13.3	62.0	63.5
11	170.5	166.4	170.4	166.0
OCH_3	52.0	51.5	52.1	51.6
1′	100.1	95.9	100.4	95.9
2′	74.7	70.5	74.6	70.5
3′	78.0	72.4	77.8	72.3
4′	71.6	68.2	71.4	68.1
5′	78.4	72.3	78.2	72.3
6′	62.8	61.7	62.6	61.6
$\underline{CH_3}CO$	-	20.1, 20.6, 20.7		20.1, 20.5, 20.7, 20.8
$CH_3\underline{CO}$	-	169.1, 169.4, 169.7,		169.0, 169.3, 169.5,
		170.1, 170.2, 170.6		170.1, 170.4, 170.5

30%. Likewise the acetate derivative showed, from the beginning, a set of extra peaks in its spectra. Close examination of COSY, HMQC and HMBC spectra allowed full assignment of these peaks to the isomeric structure (**5**) with the benzoate group shifted to C-6 OH. This structure is named isoarborside C. The ^{1}H-NMR assignments were confirmed by extensive decoupling and 1D TOCSY experiments using shaped selective pulses on H-8 of (**5**) and H-5 of (**5a**). The ^{1}H- and ^{13}C-NMR assignments of **5** and **5a** are in the Experimental. Ester interchange has been observed with 6-*O*-cinnamoyl- and 7-*O*-cinnamoyl-6β-hydroxyloganin previously isolated from *Nyctanthes arbor-tristis* leaves.

Compound **6** was identified as 6β-hydroxyloganin.[8-14] New NMR spectral data for **6** [in CD_3OD rather than D_2O (**14**)] and the completed data for the acetate (**6a**) are shown in Tables 5 and 6.

Nyctanthoside (**7**) was previously isolated from this plant,[3] however, the complete spectral data were not reported. ^{1}H- and ^{13}C-NMR spectra of **7** and its acetate (**7a**) were assigned by a combination of COSY, 1D-TOCSY, DEPT, HMQC and HMBC experiments (Tables 5 and 6).

Rengyolone (**1**) and its acetate (**1a**) possessed *in vitro* antiplasmodial activity with the IC_{50} values of 2.1 and 4.6 mg/ml, respectively, while the iridoids **2**, **4**, **6** and **7** did not exhibit any activity (IC_{50} > 20μg/ml).

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