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STUDIES ON INDIAN MEDICINAL PLANTS-PART LXXV[1]

NISHINDASIDE, A NOVEL IRIDOID GLYCOSIDE FROM VITEX NEGUNDO

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Abstract - Two new glucosidic iridoids, designated as nishindaside (2a) and negundoside (3a), have been isolated from the leaves of Vitex negundo (Verbenaceae). The absence of a C(3)-C(4) double bond and the presence of an equatorial 3-methoxy group in 2a, the 3-methoxy-3,4-dihydro derivative of agnuside (1a), were established from ^{1}H and ^{13}C NMR analyses, corroborated by mass spectral fragmentation of the hexaacetate (2b). Negundoside (3a) could be concluded to be the 2'-p-hydroxy benzoyl derivative of mussaenosidic acid from the results of alkaline hydrolysis and the shifts in the signals of C-1', C-2' and C-3' in its ^{13}C NMR spectrum.

tex negundo L. (local name Nishinda), longing to the family Verbenaceae, is puted to have antiinflammatory and tiarthritic properties in the Indian stem of medicine[2]. Earlier workers ported[3-11] the isolation of a variety constituents including the iridoid nuside (1a). Re-examination of the thanolic extract of its leaves led us two other iridoid glycosides, desig-ted as nishindaside (2a) and negundo-de (3a), the structure determination which is discussed in the present per.

Nishindaside, amorphous, $[\alpha]_D^{25}$ 3.5°, appeared to be an iridoid glyco-de from colour reactions and the similarity of its IR spectrum with that of 1a. Absence of the characteristic band at 1645-1650 cm^{-1}, however, ruled out the possibility of a -O-C(3)=C(4)- moiety, usually present in the iridoids.

Though the M^+ ion could not be detected, significant peaks at m/z 138, 121, 93 and 65 in its mass spectrum provided evidence for the presence of a monohydroxy benzoyl group in the molecule. That the compound is in fact an ester of p-hydroxy benzoic acid was evident from a symmetrical double doublet at δ 7.86 and 6.86 (J 8 Hz) in its ^{1}H NMR spectrum (Table 1) which also provided additional structural information as in the sequel.

A 3H singlet at δ 3.40 indicated a methoxy group attached to the iridoid nucleus. The doublets at δ 4.96 (J 6Hz) and 4.52 (J 8 Hz) could be assigned to two acetal protons, the chemical shift and the coupling constant of the latter being commensurate with a β-glycoside. Furthermore, a broad singlet at δ 5.76 suggested a trisubstituted double bond in the cyclopentane ring. The absence of any other olefinic proton signals, however, corroborated the absence of the C(3)-C(4) double bond.

Hydrogenation of nishindaside with Adams catalyst afforded 4, the ^{1}H NMR spectrum of which showed no signal for the vinylic proton and the aromatic moiety. Instead, a 3H doublet was observed at δ 1.08 (J 7 Hz) for a methyl group obviously formed by hydrogenolysis of an allylic ester. The above facts strongly supported the location of the trisubstituted double bond between C(7) and C(8) and the attachment of the aromatic ester group to C(10) of the iridoid nucleus.

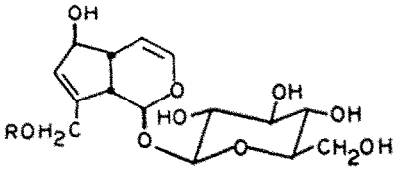
On treatment with Ac_2O/Py at room temperature, nishindaside afforded a crystalline hexaacetate (2b). Its high field (400 MHz) ^{1}H NMR spectrum exhibited five singlets in the region δ 2.0-2.1 ppm for aliphatic acetates and a more deshielded signal at δ 2.31 assignable to an aromatic one. Of the aliphatic acetoxy groups, four could be located in the sugar residue which was identified as glucose from the observed all diaxial coupling constants for 2'-H, 3'-H and 4'-H. That the remaining one was allylic to the double bond became apparent from the 1H multiplet at δ 5.60. In addition, the existence of an equatorial methoxy group at C-3 could be deduced from the double doublet at δ 4.75 ($J_{AX}+J_{BX}$=13 Hz) assigned to the axial C(3) proton.

Like the parent compound, the hexaacetate (2b) also did not show the molecular ion in the mass spectrum (CI) but the primary fragment at m/z 691 could easily be rationalised by assuming the loss of a molecule of acetic acid from $\overline{\angle M + H \rangle}^+$. Further elimination of 32 mass units, compatible with the expulsion of methanol, secured the presence of the methoxy group in the molecule. The spectrum exhibited characteristic peaks for the sugar and the aromatic ester moieties as well.

All the above evidences therefore led to structure 2a for nishindaside. It was further corroborated by ^{13}C NMR spectrometry. For this purpose, the spectrum of 1a had to be examined first. It displayed separate signals for all the carbons (except for the chemically equivalent pairs 2", 6" and 3", 5"). The individual assignments (Table 2), based on the splittings in the SFORD spectrum and chemical shift considerations, are in very good agreement with the reported data for aucubin (1b)[12] and melampyroside (1c)[13].

The chemical shifts of the carbons of 2a were found to be very close to those of 1a and the disappearance of signals at δ 141,6 and 105,4 in the spectrum of 2a was in conformity with the absence of the 3,4-unsaturation. Additional signals observed at δ 100,0 and 30,0 ppm could however be assigned to C-3 and C-4, the more deshielded one being assigned to C-3 carrying a methoxy group.

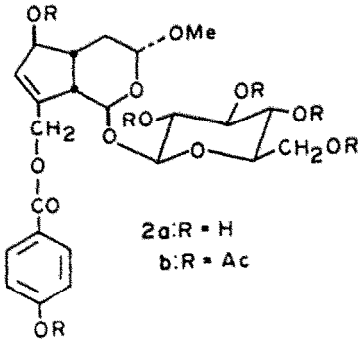
The other compound, negundoside (3a), m.p. 160-64°, $\overline{\angle \alpha \rangle}_D^{25}$ -130.0°, could be inferred to be an αβ-unsaturated 4-carboxy iridoid carrying a p-disubstituted aromatic ring from the characteristic ^{1}H NMR signals.



1a : R = COC_6H_4OH (p)

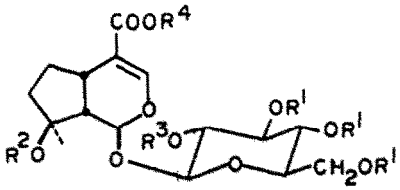
b : R = H

c R = COC_6H_5



2a:R = H

b:R = Ac



3a: $R^1 = R^2 = R^4 = H$, $R^3 = COC_6H_4OH$ (p)

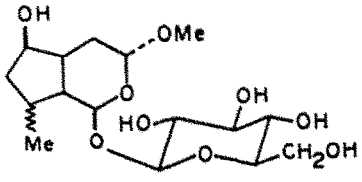
b: $R^1 = R^2 = R^3 = R^4 = H$

c: R^1 = Ac, $R^2 = R^4 = H$, $R^3 = COC_6H_4OH$ (p)

d: R^1 = Ac, $R^2 = R^4 = H$, $R^3 = COC_6H_4OAc$ (p)

e: R^1 = Ac, $R^2 = H$, $R^3 = COC_6H_4OMe$ (p), R^4 = Me

f: $R^1 = R^2$ = Ac, $R^3 = COC_6H_4OAc$ (p), $R^4 = H$



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Treatment of 3a with methanolic sali at room temperature afforded thyl-p-hydroxy benzoate and mussaenidic acid (3b)[14]. The linkage of the matic ester moiety at position 2' established from a downfield shift C-2' and shielding of C-1' and C-3' nals in the ^{13}C NMR spectra of 3a compared to 3b.

Since the structure of probably identical compound has recently n reported[15], only the additional ormation related to the structure cidation is presented herein.

Acetylation of 3a with Ac_2O/Py at m temperature in our hands yielded riacetate (3c) besides the tetratate (3d)[15]. The former furnished a ethyl derivative (3e) which exhibitwo 3H singlets at δ 3.84 and 3.40 the ^1H NMR spectrum corroborating reby the presence of a phenolic and arboxylic group in negundoside (3a).

On the other hand, the acetylation of 3a at higher temperature led to a pentaacetate (3f). The downfield shift of the methyl, 1-H and 9-H signals in its ^1H NMR spectrum over those of 3d adduced evidence for the β-oriented tertiary hydroxyl group[16] in negundoside (3a). The same conclusion could also be arrived at from the chemical shift (δ 52.2) of C-9 in the ^{13}C NMR spectrum of 3a [cf.[17] δ 50 ± 2 ppm for C_8-OH (β) and 44.5 ± 1 ppm for C_8-OH(α)].

Nishindasside is a unique 3,4-dihydro C_9-iridoid carrying a 3-methoxy group hitherto not encountered in nature, while negundoside belongs to the rare 2'-esterified iridoids, the only other compounds being reported very recently from Scrophulariaceae[18] and Rubiaceae[19].

Table 1

^{1}H NMR Spectral data of the iridoid glycosides[a,b]

Position	1a	2a	2b	3a	3b	3c	3d	3e	3f
1		4.96d (6)	5.21d (3)	5.40d (4)	5.36d (4)				5.80brs
3	6.36dd (2,7)		4.75dd (6,7)	7.04s	7.28s	7.12s	7.04s	7.10s	7.10s
4	5.10dd (4,7)								
5	2.90m		2.65m	2.70m			2.80m	2.90m	2.80m
6	4.38m		5.60m						
7	5.80brs	5.76brs	5.86brs						
9	2.90m		3.15brd (9)	2.06dd (3,9)	2.10dd (4,9)	2.26dd (4,11)		2.28dd (4,10)	2.65brd (8)
10	4.90brs	4.84brs	4.92, 4.98[c]	1.14s	1.20s	1.26s	1.24s	1.28s	1.44s
1'	4.54d (8)	4.52d (8)	4.88d (8)	4.85d (8)	4.56d (8)	5.00d (8)	4.96d (8)	5.00d (8)	4.98d (8)
2'			5.02dd (8,10)	4.75t (8)		5.10-5.50m	5.10-5.50m	5.10-5.50m	5.10-5.60m
3'			5.08t (10)			5.10-5.50m	5.10-5.50m	5.10-5.50m	5.10-5.60m
4'			5.28t (10)			5.10-5.50m	5.10-5.50m	5.10-5.50m	5.10-5.60m
5'			3.70ddd (2,5,10)				3.85brd (10)		3.86brd (10)
6'	3.7m		4.05dd (2,13) 4.19dd (5,13)			4.26m	4.26m	4.26m	4.20dd (2,12) 4.46dd (5,12)
2",6"	7.90d (8)	7.86d (8)	8.10d (9)	7.80d (8)		7.80d (9)	8.01d (8)	7.90d (8)	8.01d (8)
3",5"	6.90d (8)	6.86d (8)	7.20d (9)	6.85d (8)		6.80d (9)	7.11d (8)	6.90d (8)	7.10d (8)
OCH_3		3.40s	3.41s					3.4s 3.84s	
$OCOCH_3$			2.00-2.10, 5xs 2.31s			1.95s 2.06s 2.12s	1.96s 2.04s 2.12s 2.36s	1.90s 2.06s 2.10s	1.95s 1.98s 2.04s 2.12s 2.36s

[a] δ (ppm) downfield from TMS, J values in parentheses.

[b] Solvents: 1a, 2a, 3a, 3b in DMSO-d_6; 2b, 3d, 3e, 3f in $CDCl_3$ and 3c in $CDCl_3$-CD_3OD.

[c] Inner limbs of an ABq, outer limbs merged with other signals.

Table 2

^{13}C NMR chemical shifts in CD_3OD

rbon	1a	2a	3a	3b
	97.9	98.4	94.9	95.1
	141.6	100.0	151.0	151.6
	105.4	30.2	113.5	*
	46.1	44.6	30.9	31.8
	82.7	80.8	30.0	30.5
	132.3	131.4	41.2	40.7
	142.7	142.5	79.7	80.4
	48.4	49.2	52.2	52.1
	63.5	63.0	24.3	24.5
	-	-	169.8	*
	100.1	100.0	97.6	99.6
	74.7	74.6	75.8	74.5
	78.0	77.9	74.7	78.0
	71.3	71.2	71.6	71.5
	77.8	77.9	78.1	77.7
	62.6	62.6	62.6	62.7
	121.9	121.8	122.0	-
,6"	132.7	132.7	132.7	-
,5"	116.2	116.1	116.0	-
	163.5	163.3	163.0	-
H_3	-	56.1	-	-
)	167.7	167.6	167.1	-

'he quaternary carbon signals not
)served probably due to high relaxation
me.

EXPERIMENTAL

All melting points are uncorrected. ! spectra were recorded on a Perkin-.mer infrared spectrophotometer (model '7) and mass spectra (EI) on a Hitachi IU-6L instrument. ^1H and ^{13}C NMR spectra :re mostly measured on a Jeol FX-100 FT IR spectrometer using TMS as internal :andard.

Isolation of the iridoid glyco-.des. Dried leaves (5 kg) of Vitex egundo were successively extracted in a oxhlet with petrol, $CHCl_3$ and MeOH. The ethanolic extract was concentrated to a yrup, triturated with H_2O and the queous layer exhaustively extracted with -BuOH. The residue (400 g) obtained fter complete evaporation of BuOH was chromatographed repeatedly over silica gel, eluting with $CHCl_3$ with increasing MeOH content. The first few fractions eluting with 8% MeOH/$CHCl_3$ were pooled; evaporation gave pure agnuside (1a). The subsequent fractions on evaporation gave a mixture of 1a and 2a from which 2a was purified by prep. TLC. The 10% MeOH/$CHCl_3$ eluents afforded compound 3a.

Agnuside (1a). Recrystallisation from MeOH-$CHCl_3$ yielded agnuside (3 g), m.p. 148-52°, IR (nujol): ν_{max} 3400, 1708, 1640, 1590, 1455 cm^{-1}.

Nishindaside (2a). Obtained as an amorphous powder (150 mg), $[\alpha]_D^{25}$ -83.5° (MeOH, c 0.974), IR(KBr): ν_{max} 3400(OH), 1700(CO), 1605, 1590, 1450 (aromatic)cm^{-1}.

Hexa-O-acetyl derivative of 2a. Compound 2a (25 mg) was treated with dry pyridine (0.1 ml) and Ac_2O(0.1 ml) at room temp and left overnight. After addition of 0.1 ml MeOH, the solution was left for 20 min, then evaporated to give 2b (22 mg), crystallised from $CHCl_3$-petrol as needles, m.p. 161-62°, $[\alpha]_D^{25}$ -71.4°($CHCl_3$, c 0.68), IR(KBr): ν_{max} 1740 cm^{-1}; CI-MS (Isobutane): m/z (rel.int.%) 691(1), 659(2), 331(97), 271(95),229(37), 181(97), 169(92), 162(98), 139(97), 121(80), 109(39), 57(100).

Hydrogenation of 2a. Compound 2a (20 mg) was hydrogenated using PtO_2 (2 mg) in 4 ml EtOH until H_2 uptake ceased. After usual work up and purification, 4 (15 mg) was obtained as an amorphous material.

Negundoside (3a). Crystallised from MeOH-$CHCl_3$ as white needles (1g), m.p. 160-64°, $[\alpha]_D^{25}$ -130° (MeOH, c 0.122), IR(nujol): ν_{max} 3400(OH), 1710, 1690(CO), 1640, 1605, 1590 and 1455 cm^{-1}.

Acetylation of 3a at room temp. Compound 3a (100 mg) was acetylated with pyridine (0.5 ml) and Ac_2O (0.5 ml) at room temp overnight. Usual work up followed by purification through column chromatography yielded the products 3c (40 mg) and 3d (35 mg).

Tri-O-acetyl derivative of 3a. Compound 3c, amorphous, $[\alpha]_D^{25}$ -91.6° (MeOH, c 0.936), IR(nujol): ν_{max} 3350, 1740, 1710, 1620 and 1230 ($OCOCH_3$)cm^{-1}.

Tetra-O-acetyl derivative of 3a. 3d, m.p. 118-20°, $[\alpha]_D^{25}$ -96.6° ($CHCl_3$, c 0.17), IR ($CHCl_3$): ν_{max} 1740, 1710 (CO), 1635, 1600 and 1240 ($OCOCH_3$) cm^{-1}.

Penta-O-acetyl derivative of 3a. Compound 3a (60 mg) was acetylated with pyridine (0.3 ml) and Ac_2O (0.3 ml) at 100° for one hr and worked up in the usual way. Purification by column chromatography yielded two products 3d (20 mg) and 3f (30 mg). Compound 3f, an amorphous powder, $[\alpha]_D^{25}$ -48.4° ($CHCl_3$, c 0.64); IR ($CHCl_3$): ν_{max} 1745, 1720, 1695, 1630, 1600, 1230 ($OCOCH_3$) cm^{-1}.

Methylation of 3c. Treatment of 3c (20 mg) with CH_2N_2 overnight afforded the amorphous compound 3e (15 mg), $[\alpha]_D^{25}$ -57.6° ($CHCl_3$, c 0.26), IR (nujol): ν_{max} 3400, 1740 (CO), 1690, 1635, 1600 and 1230 ($OCOCH_3$) cm^{-1}.

Hydrolysis of 3a. Compound 3a (50 mg) was hydrolysed by keeping it overnight with 0.1N NaOH (1 ml) and then neutralising by passing CO_2. Purification by column chromatography afforded methyl-p-hydroxy benzoate and compound 3b (30 mg).

Mussaenoside tetraacetate and pentaacetate. Compound 3b (50 mg) was methylated with CH_2N_2 overnight and the crude material acetylated with Ac_2O/Py at 100°. Usual work up followed by chromatographic purification yielded mussaenoside tetraacetate (20 mg) and mussaenoside pentaacetate (15 mg), identified by direct comparison (co TLC, IR, ^1H NMR) with authentic specimen.

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