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Short Communication

Benzoxazinoids from *Acanthus illicifolius* †

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Abstract

1,4-Benzoxazinone derivatives identified as (2*R*)-2-β-D-glucopyranosyloxy-2*H*-1,4-benzoxazine-3(4*H*)-one(GHBOA, blepharin) (**1**) and (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazine-3-one (GDIBOA) (**2**) have been isolated from the pods of a mangrove *Acanthus illicifolius*. The crude methanolic extract of this plant exhibited analgesic and anti-inflammatory activities. Structures of these compounds have been established on the basis of their spectral data and the data recorded on their acylated derivatives. Data reported earlier for pentacetyl blepharin has also been discussed.

Keywords: Benzoxazinoids, *Acanthus illicifolius*, anti-inflammatory, analgesic, blepharin.

Several derivatives with 1,4-benzoxazine-3-one skeleton have been found to occur in the form of β-D-glycosides as allelochemicals in different species of *Graminae*,[1–3] *Acanthaceae*,[4] *Ranunculaceae*[5] and *Scrophulariaceae*.[6] The role of heterocyclic aglucones, specially DIBOA (2,4-dihydroxy-2*H*-1,4-benzoxazine-3-one), isolated from *Secale cereale*,[7] *Acanthus mollis*[8] and *Consolida orientalis*[5] and its 7-methoxy derivative or DIMBOA, also isolated from *Graminae* species[9] as plant resistant factors aganist microbial disease and insects, is well known.[10–11] The biological function of such glucosides as possible endogenous ligands in the plant cell has also been investigated.[12,13] The glucosides are also reported to inhibit prostate cancer cell lines.[14] Due to their biological activity, these compounds are continuously under investigation. As part of our search for biologically active substances from mangrove, the crude methanolic extract of *Acanthus illicifolius* exhibited significant analgesic and anti-inflammatory activities.[15] In our earlier communication, we have reported the isolation of benzoxazoline-2-one[16] and a preliminary study on the glycosides (**1**) and (**2**) as the analgesic and anti-inflammatory principle of this mangrove.[17] We report herein the details on identification of the glucosides on the basis of their spectral data and of their peracylated derivatives and of respective aglucones and benzoxazolin-2-one on the basis of DCI-MS data and comment on the data reported earlier by Chatterjee *et al*.[18] on pentacetyl derivative of blepharin.

There have been a number of phytochemical investigations on this mangrove.[19–24] However, this is the first record of the presence of both the glucosides, blepharin (**1**), GDIBOA (**2**) and of respective aglucones (**3**) and (**4**) in this species belonging to *Acanthaceae.*

† Dedicated to Prof. S. C. Bhattacharyya.
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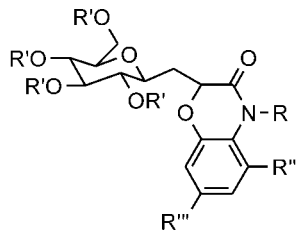
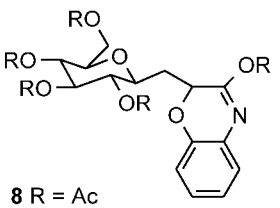
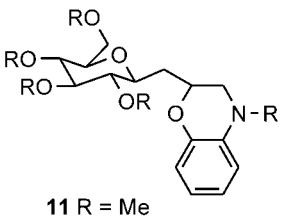


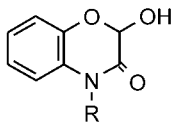
1 R =R' = R'' = R''' = H
2 R = OH, R' = R'' = R''' = H
6=7 R' = Ac; R =R'' = R''' = H
9 R = H, R' = Ac; R'' = Cl; R''' = OCH_3
11 R = OH; R' = Ac; R'' = R''' = H



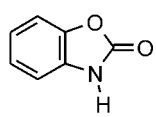
8 R = Ac



11 R = Me



3 R = H
4 R = OH



5

n-Butanol fraction on chromatography over silica gel with gradient MeOH:$CHCl_3$ yielded a solid, m.p. 214°C, which looked homogeneous on TLC but was found to be unstable. Hence, it was subjected to DCIMS with NH_3, ND_3 and CH_4 as the reagent gases. NH_3/DCIMS spectrum of the solid showed prominent peaks as ammonium adducts of blepharin (**1**) and GDIBOA (**2**), at *m/z* 345 (90) and 361(base peak) corresponding to M^+ 327 and M^+ 343, respectively. The spectrum also exhibited intense peaks at *m/z* 198 $(C_6H_{12}O_6+NH_4)^+$, 180 $(C_6H_{10}O_5+NH_4)^+$ and 162 $(C_6H_8O_4+NH_4)^+$ by consecutive elimination of water molecule from the carbohydrate moiety supporting glucosidic nature of these compounds. With deuterated ammonia, ND_3, the peaks at *m/z* 345 and 361 were shifted to *m/z* 349 and 365 with cluster of peaks from *m/z* 349 to 354 and *m/z* 365 to 370. These are indicative of the presence of five acidic hydrogens in both the glucosides. The acidic hydrogens get exchanged with deuterium in the ion source when deuterated ammonia is employed as the reagent gas.[25]

Methane /DCIMS further supported the presence of (**1**) and (**2**) with $(M_1+H)^+$ and $(M_1+C_2H_5)^+$ at *m/z* 328 and 356 and signals at *m/z* 344 and 372 for $(M_2+H)^+$ and $(M_2+C_2H_5)^+$, respectively. The curious feature of DCI spectra is the presence of intense signals at *m/z* 198, 183 and 153 in the NH_3/DCIMS spectrum and corresponding peaks in methane/DCI spectrum. This reveals the presence of the aglucones HBOA (M^+165) (**3**), DIBOA (M^+ 181) (**4**) and BOA (M^+ 135) (**5**). These must have originated due to the unstable nature of glucosides. Glucosides are known to be heat sensitive and to undergo autolysis to give aglucones and ultimately BOA.[2,7]

Compound **1**, m.p. 220, M^+ 327, $C_{14}H_{17}O_8N$, λ_{max} (MeOH) 238 nm with shoulders at 273 and 282 nm. IR ν_{max} (Nujol) 3320, 1680, 1660, 1600, 1455, 1375, 1300, 1270, 1210, 1150, 1130, 1070, 1020, 980, 880, 840, 830. ^{1}H NMR (200 MHz, D_2O): δ 5.83 (1H, s, H-2), δ 4.55 (1H, d, J=7.7 Hz), '3.0–3.8 (6H, m); 6.95 (3H, m)' 7.3 (1H, brs) and 10.94 (1H, brs). ^{13}C NMR (Table I) of **1** exhibited, as expected, 14 signals that could be assigned to all the carbon atoms of blepharin. Multiplicity of these signals was determined on the basis of DEPT experiments.

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Table I
^{13}C NMR of compounds 1 and 2 in D_2O and 6, 8 and 11 in $CDCl_3$

C	1	6	8	2	11
C-2	94.49	96.0	100.85	99.3	97.0
C-3	160.36	160.4	172.19	158.5	154.9
C-5	115.4	115.9	118.96	116.7	113.9
C-6	123.7	124.2	126.25	126.4	123.6
C-7	123.1	123.4	123.32	124.9	123.61
C-8	117.6	117.8	121.32	119.9	117.5
C-9	126.7	124.9	141.92	128.1	125.02
C-10	140.27	140.1	140.51	143.1	140.5
C-1′	103.46	100.6	97.28	104.5	100.39
C-2′	73.2	71.06	70.99	75.5	71.06
C-3′	77.3	72.5	72.41	79.02	72.49
C-4′	70.29	68.1	67.99	71.8	68.1
C-5′	76.7	72.3	72.32	78.14	72.49
C-6′	60.9	61.8	61.73	63.2	61.7
– CH_3C=O		20.4	20.43		20.5
		20.4	20.43		20.5
		20.6	20.43		20.7
		20.6	20.62		20.7
N=C-$OCOCH_3$			28.25		
		169.2	168.99		169.2
		169.2	169.11		169.47
		170.1	169.34		170.15
		170.5	170.30		170.7
N=C-$OCOCH_3$			161.9		

The EIMS of (**1**) is characterized by M^+327 (2.7), and fragments at *m/z* 166 (17), 165 (40), 149 (30), 148 (26.8), 137 (37.8), !36 (100, base peak), 120 (19.6), 109 (12), 85 (15.5), 80 (9.5), 73 (16.7).

Peracylation of (**1**) with Ac_2O/pyridine yielded a tetraacetate (**6**), M^+ 495. IR ν_{max} (KBr) 1750, 1230 and 1710 cm^{-1} characteristic of carbonyl stretching vibrations of acetates and the amide. Its ^{1}H NMR (100 MHz, $CDCl_3$; Table II) showed the presence of acetate groups at δ 1.92 (3H, s), 1.96 (6H, s) and 2.02 (3H, s). Signal due to amide proton was apparent at δ9.35 (1H, s); sugar protons were evident at δ3.7–5.3 (6H, m) and the singlet at δ5.6 could only be assigned to H-2. The coupling constant of the anomeric proton in (**1**) at δ4.55 (d, *J*=7.7 Hz) and at δ 4.92 (d, *J*=8Hz) in (**6**) was suggestive of a β-glucopyranoside. Multiplet due to four aromatic protons appeared between δ6.78 and 7.02. Chemical shift values of H-2 of (**1**) and (**6**) point to the 2*R* configuration of stereogenic center C-2.[25] The EIMS of (**6**) exhibited M^+ at *m/z* 495 with fragments at *m/z* 331, 271, 169, 148, 127, 120, 97, 80, 43 (base peak).

A comparison of ^{1}H NMR data of **6** (Table II) with the reported values[18, 26–27] shows some discrepancy, as evident from the table. The data obtained by us on blepharin acetate (**6**) are well in agreement with the one reported by Tietze *et al*.[26] for blepharin acetate (**7**) synthesized by treatment of 2-bromo-2*H*-1,4-benzoxazin-3(4H)-one with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in the presence of mercury cyanide $Hg(CN)_2$. It is at variance with that of Chatterjee *et al*.[18] who reported the formation of a pentacetate (**8**) with the C-3 amido carbonyl of blepharin in enol form

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Table II
Comparison of ^{1}H NMR data in $CDCl_3$ of blepharin acetate (δ values)

H	**6**	**7**	**8**	**9**	**11**
H-2	5.6 (s)	5.64 (s)	Not reported	5.63 (s)	5.8 (s)
Aromatic (4H, m)	6.78–7.02	6.84–7.1	6.79–7.4	6.67–6.95	7.07–7.4
Sugar protons	3.78–5.16	3.8–5.2	3.7–5.14	4.0–5.3	3.7–5.06
Anomeric	4.92 (1H, d, J = 8Hz)	5.01 (1H, d, J = 8Hz)	4.79 (1H, d, J = 7.8Hz)	5.22 (1H, d)	4.95 (1H, d, J = 8Hz)
-NH	9.35 (brs)	9.15 (brs)	—	9.8 (brs)	—
-O-CO-CH_3	2.02 (s) 1.96 (s) 1.96 (s) 1.92 (s)	1.99 (s) 2.02 (s) 2.04 (s) 2.09 (s)	1.91 (s) 1.95 (s) 1.96 (s) 2.03 (s)	1.929 (s) 1.989 (s) 1.998 (s) 2.068 (s)	2.02 (s) 2.06 (s) 2.06 (s) 2.12 (s)
N=C-OCOCH_3			2.69 (s)		

getting acetylated. The IR spectrum of pentacetate shows absorption at 1755 and 1740 cm^{-1} which is attributed to acetyl carbonyls; tetracetyl derivative exhibits corresponding absorption at 1755 and 1710 cm^{-1} which have been attributed to acetyl and amide carbonyl, respectively.

Chatterjee *et al*.[18] also report methylation with dimethyl sulfate and alkali yielding a pentamethyl derivative (**10**) wherein amide proton also gets methylated. The signal at δ 3.35 in the ^{1}H NMR of pentamethyl derivative has been assigned to methyl attached to nitrogen, whereas one proton singlet at δ 5.76 is assigned to H-2.

Compound (**2**), a crystalline solid, m.p. 258–260°C (decomposition). UV λ_{max} (MeOH) 245 nm with shoulders at 276 and 285 nm. IR ν_{max}(nujol) showed peaks of variable intensities at 3340, 2900, 1640, 1610, 1510, 1440, 1320, 1290, 1230, 1060, 1025, 1000 and 860 cm^{-1}. Its ^{1}H NMR (200 MHz; D_2O) showed four aromatic protons as multiplet between δ 7.1 and 7.4. C-2 methine appeared as a broad singlet at δ 5.9. The six sugar protons were evident as multiplets in the region δ 3.1–3.9 with anomeric proton at δ 4.8 (d, J=7.7Hz). Its ^{13}C NMR was similar to compound **1** (Table I). Its EIMS showed M^+ 343, besides fragments at 327, 181, 165, 149, 148, 136 (base peak), 135, 109, 108, 73. It is evident from the spectral data that compounds (**1**) and (**2**) have closely related structures, but differs in molecular weight by 16 amu. Compound (**2**) must therefore contain an extra -OH group. Measurement of UV absorption, after the addition of 0.1 N NaOH and 0.1 N HCl showed neither a bathochromic nor hypsochromic shift, suggesting that -OH group is not phenolic and should replace amide proton of blepharin. It is assigned structure (**2**). Further, confirmation of the structure was established by the spectral data of peracylated derivative. Acetylation of (**2**) yielded a tetraacetate with ^{1}H NMR and ^{13}C NMR data, as given in Tables I and II, well in agreement with the structure (**11**) assigned to it. Its EIMS further confirmed the structure with a M^+ at *m/z* 511 and fragments at *m/z* 331, 271, 169 (base peak), 109, 43. Spectral data not recorded earlier by other investigators have been included.

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