1260, 1230, 1190, 1175, 1155, 1135, 1115, 1060, 1025w, 980, 955, 935w, 875, 865sh, 845sh, 800, 780, 725, 710; ^{1}H NMR: δ2.38 (3H, *s*, Me-3), 3.97 (3H, *s*, MeO-7), 6.30 (1H, *s*, H-8), 6.84 (1H, *s*, H-4), 12.75 (1H, *s*, OH-5), 14.28 (1H, *s*, OH-10), addition of D_2O removes the OH signals at δ12.75 and 14.75; UV λ_{max}^{MeOH} nm (log ε): 255 (4.25), 312 (3.55), 330 inf. (3.46), 508 inf. (3.72), 536 (3.77), 576 (3.72), 612 (3.49).

1,5,10-*Trihydroxy*-7-*methoxy*-3-*methyl*-1H-*naphtho*-[2-3-c]-*pyran*-6,9-*dione* (**2**). MS *m/z* 304 (31.4%), 289 (11%), 261 (100%), $C_{15}H_{12}O_7$ requires 304.0581, found 304.0590; deep purple crystals (Me_2CO) mp 245° on a preheated block; IR ν_{max}^{KBr} cm^{-1}: 3425*s*, 1600, 1580, 1440, 1400, 1385sh, 1280sh, 1250, 1225sh, 1190w, 1155, 1110w, 1070, 1040, 990, 955, 890, 860*br*, 805; ^{1}H NMR $CDCl_3$: δ2.18 (3H, *s*, Me-3), 3.94 (3H, *s*, MeO-7), 6.26 (1H, *s*, H-8), 6.45 (1H, *s*, H-4), 6.73 (1H, *s*, H-1), 12.85 (1H, *s*, OH-5), 13.25 (1H, *s*, H-10), addition of D_2O removes the OH signals at δ12.85 and 13.25; $(CD_3)_2$SO: δ2.04 (3H, *s*, Me-3), 3.84 (3H, *s*, MeO-7), 6.10 (1H, *s*, H-8), 6.20 (1H, *s*, H-4), 6.55 (1H, *d*, J = 6, H-1), 6.82 (1H, *d*, J = 6, OH-1), 12.76 (1H, *s*, OH-5), 13.22 (1H, *s*, OH-10). Addition of D_2O removes the OH signals at δ6.82, 12.76, 13.22, and the doublet at 6.55 appears as a singlet at 6.63; UV λ_{max}^{MeOH} nm (log ε): 228 (4.06), 263 (4.05), 278sh (4.01), 343 (3.23), 509 (3.80), 535 (3.87), 572 (3.71).

5,10-*dihydroxy*-1,7-*dimethoxy*-3-*methyl*-1H-*naphtho*-[2,3-c]-*pyran*-6,9-*dione* (**3**). MS *m/z* (rel. int.): 318 (34.7), 287 (88.9), 286 (100); $C_{16}H_{14}O_7$ requires 318.0738, found 318.0764; purple crystals (C_6H_6) mp 190° on a preheated block; IR ν_{max}^{KBr} cm^{-1}: 1605, 1585, 1430, 1380, 1310w, 1250, 1220, 1185, 1150, 1080, 1050, 1015, 955, 885w, 865w, 815, 790sh; ^{1}H NMR $CDCl_3$: δ2.15 (3H, *s*, Me-3), 3.62 (3H, *s*, MeO-1), 3.93 (3H, *s*, MeO-7), 6.24 (1H, *s*), 6.25 (1H, *s*), 6.29 (1H, *s*), 12.80 (1H, *s*, OH-5), 13.24 (1H, *s*, OH-10), addition of D_2O removes signals at δ12.80 and 13.24; UV λ_{max}^{MeOH} nm (log ε): 228 (4.01), 263 (3.98), 278sh (3.96), 342 (3.15), 507 (3.68), 535 (3.75), 572 (3.63).

2,3-*Dihydro*-5-*hydroxy*-8-*methoxy*-2,4-*dimethyl*-*naphtho*-[1,2-b]-*furan*-6,9-*dione* (**4**). MS *m/z* (rel. int.): 274 (100), 259 (23), 245 (53), $C_{15}H_{14}O_5$ requires 274.084, found 274.087; red crystals (EtOAc) mp 220–224°; IR ν_{max}^{KBr} cm^{-1}: 1665, 1625, 1585, 1460, 1435, 1405, 1380sh, 1350w, 1270w, 1240, 1210, 1190, 1160, 1105w, 1085, 1045, 1025, 1010sh, 960, 935, 875, 860, 810, 790, 760, 725; ^{1}H NMR: δ1.59 (3H, *d*, J = 6, Me-2), 2.25 (3H, *s*, Me-4), 2.75 (1H, *dd*, J = 17, 6.5, H-3), 3.30 (1H, *dd*, J = 17, 9, H-3), 3.88 (3H, *s*, MeO-8), 5.20 (1H, *m*, H-2), 6.06 (1H, *s*, H-7), 13.50 (1H, *s*, OH-5) addition of D_2O removes the OH signal at δ13.50; UV λ_{max}^{MeOH} nm (log ε): 225 (4.70), 299 (4.03), 480 (3.99), 500 (3.97), 532 (3.63).

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A BIANTHRONE *C*-GLYCOSIDE FROM *ASPHODELUS RAMOSUS* TUBERS

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Key Word Index—*Asphodelus ramosus*; Liliaceae; *C*-glycosides; bianthrones; ^{13}C NMR.

Abstract—Ramosin, the first member of the new class of bianthrone *C*-glycosides, has been isolated from tubers of *Asphodelus ramosus*. On the basis of spectral evidence, its structure was established as (−)-10′-*C*-[β-D-glucopyranosyl]-1,1′,8,8′,10,10′-hexahydroxy-3,3′-dimethyl-10,7′-bianthracene-9-9′-dione. The complete ^{13}C chemical shift assignment of the compound is reported.

INTRODUCTION

In connection with our studies [1, 2] on Liliaceae metabolites we have investigated the components of *Asphodelus ramosus* tubers. From the ethereal extract of these tubers we isolated several bianthrone *C*-glycosides. In this paper we describe the isolation and structural determination, obtained only on the basis of spectroscopic evidence, of the major metabolite **1**, named ramosin.

RESULTS AND DISCUSSION

The negative-ion FAB-mass spectrum of **1** showed a pseudomolecular ion peak at *m/z* 671 $[M-H]^-$ that,

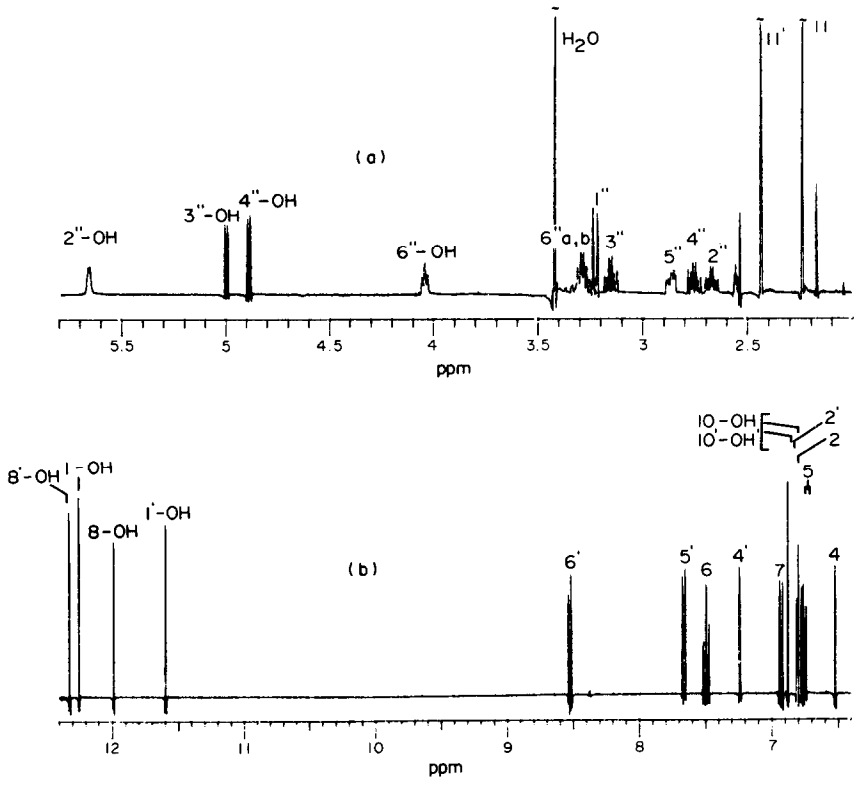
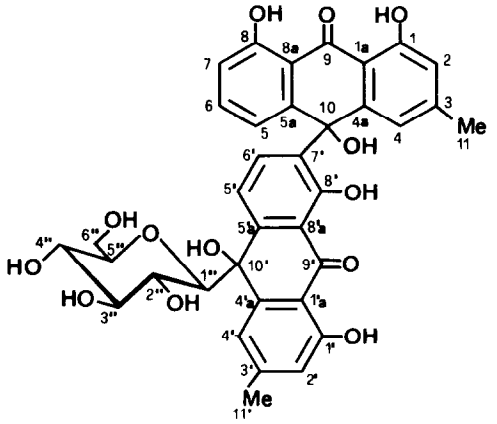


Fig. 1. Resolution-enhanced 400.135 MHz ^{1}H NMR spectrum of ramnosin **1** in DMSO-d_6: (a) δ2.0–5.8 region; (b) δ6.4–12.4 region.

together with ^{13}C NMR data, suggested the molecular formula $C_{36}H_{32}O_{13}$, indicating the presence of 21 sites of unsaturation in the structure. Ten signals due to exchangeable protons were identified in the ^{1}H NMR spectrum at 400 MHz in DMSO-d_6 (Fig. 1) by irradiation of the H_2O resonance in a saturation transfer difference spectroscopy experiment [3]. Four of these signals, appearing as singlets between δ11.6 and 12.3, may be assigned to chelated phenolic protons. The other ones, two of which appeared as singlets at δ6.88 and δ6.80, three as doublets at δ5.65 (J^3 = 4.0 Hz), δ4.99 (J^3 = 5.0 Hz) and δ4.88 (J^3 = 5.8 Hz) and one as a triplet at δ4.03 (J^3 = 5.5 Hz), may be assigned to alcoholic protons on tertiary, secondary and primary carbons, respectively. Accordingly, the ^{1}H NMR spectrum showed signals attributable to five carbinol protons in the region δ3.4–2.6, where the signals of two additional protons linked to oxygen-bearing carbons were also displayed.



1

The scalar connectivity established between the above seven protons by homonuclear decoupling experiments (Table 1), the multiplicity of their signals and the $^3J_{H,H}$ values, suggested the presence of a *C*-β-glucopyranosyl moiety in **1**. In agreement with this suggestion were the lack of signals in the anomeric region of both the ^{1}H and the ^{13}C NMR spectra and the occurrence of the peak at *m/z* 509 $[M-H-162]^-$, indicating the loss of a hexose unit, in the negative-ion FAB-mass spectrum.

In the aromatic region between δ8.6–6.5 the signals of nine protons are displayed and at δ2.23 and 2.43 appear two 3H singlets assignable to two methyl groups linked to an aromatic ring. The arrangement of these protons was easily deduced by homonuclear decoupling and COSY long-range [4] experiments (Table 1), that indicated the presence of three vicinal protons (δ6.93, 7.49, 6.75), two *ortho* protons (δ8.52, 7.66) and two pairs of *meta* protons separated by a methyl group (δ7.24, 6.81, 2.43 and 6.52, 6.77, 2.23).

The ^{13}C NMR data (Table 1), obtained by on-resonance and DEPT [5] experiments, were accounted for by 19 quaternary, 14 methine, one methylene and two methyl carbons. On the basis of chemical shifts, the presence of a *C*-glycopyranoside unit was confirmed by five oxygen-bearing methine carbon signals in the δ85–70

Table 1. Carbon–proton and proton–proton shift correlations in **1** (DMSO-d_6)*

Position	Carbon chemical shifts	DEPT	Chemical shift (coupling constants) of the one-bond heterocorrelated protons †	Chemical shift (position) of the long-range heterocorrelated protons
1	161.7	C	—	12.24 (1-OH)
1a	112.4	C	—	12.24 (1-OH), 6.77 (2), 6.52 (4)
2	116.9	CH	6.77 br s	6.52 (4), 2.23 (11)
3	148.8	C	—	2.23 (11)
4	120.5	CH	6.52 br s	2.23 (11)
4a	149.2	C	—	—
5	119.4	CH	6.75 d (8.0)	6.93 (7)
5a	149.0	C	—	7.49 (6)
6	137.1	CH	7.49 t (8.0, 8.0)	—
7	116.4	CH	6.93 d (8.0)	6.75 (5)
8	161.4	C	—	12.31 (8-OH), 7.49 (6)
8a	114.5	C	—	12.31 (8-OH), 6.93 (7), 6.75 (5)
9	193.0‡	C	—	—
10	69.9	C	—	8.52 (6′), 6.75 (5), 6.52 (4)
11	22.0	Me	2.23 s	6.77§ (2), 6.52§ (4)
1′	161.2	C	—	11.59 (1′-OH)
1′a	113.8	C	—	11.59 (1′-OH), 7.24 (4′), 6.81 (2′)
2′	117.0	CH	6.81 br s	7.24 (4′), 2.43 (11′)
3′	147.5	C	—	2.43 (11′)
4′	119.8	CH	7.24 br s	6.81 (2′), 2.43 (11′)
4′a	146.1	C	—	3.22 (1″)
5′	116.7	CH	7.66 d (8.5)	—
5′a	147.0	C	—	8.52 (6′), 3.22 (1″)
6′	132.3	CH	8.52 d (8.5)	—
7′	133.9	C	—	11.98 (8′-OH), 7.66 (5′)
8′	157.1	C	—	11.98 (8′-OH), 8.52 (6′)
8′a	114.5	C	—	11.98 (8′-OH), 7.66 (5′)
9′	192.6‡	C	—	—
10′	74.9	C	—	7.66 (5′), 7.24 (4′), 3.22 (1″)
11′	22.2	Me	2.43 s	7.24 (4′), 6.81 (2′)
1″	84.7	CH	3.22 d (9.2)	—
2″	72.0	CH	2.66 t (9.2, 9.2)	—
3″	78.2	CH	3.14 t (9.2, 9.2)	—
4″	70.1	CH	2.75 t (9.2, 9.2)	—
5″	81.1	CH	2.86 m	—
6″	61.3	CH_2	3.29 (5.0, 11.6) 3.24 (3.0, 11.6) AB of ABX	—

*In the ^{1}H NMR spectrum of the sample utilized for the 2D experiments the signals of the alcohol hydroxy protons were missing.

†Proton correlations revealed by COSY long-range and homonuclear decoupling experiments are indicated by solid lines.

‡Interchangeable values.

§Correlations obtained by choosing a delay corresponding to a 7 Hz C, H coupling.

region and one hydroxymethylene carbon at δ61.3. The presence of two carbonyl groups (δ192.6, 193.0) suggested, in addition to FAB-mass spectrometry and ^{1}H NMR [6], a bianthrone *C*-glycoside structure. Accordingly [6], UV absorption maxima (MeOH) were displayed at 262, 270, 304, 373 nm.

The full assignment of the ^{13}C NMR signals (Table 1), established on the basis of one-bond and long-range 2D-NMR heterocorrelated experiments [7], clearly indicated the structure **1** for the compound. In particular, valuable correlations were those found for the quaternary carbinol carbons at δ69.9 and 74.9. Actually, the carbon at δ69.9 was correlated with the protons at positions 5 and 4, the former belonging to the group of three vicinal aromatic protons and the latter belonging to one of the two *meta*-proton systems. In addition, this carbon was also correlated with the 6′-H, whose *ortho* 5′-proton was correlated with the other carbinolic quaternary carbon at 74.9 ppm. This latter carbon, besides the correlation with the 4′-H belonging to the other *meta*-proton system, showed connection with the 1″-H, thus showing the location of the saccharide unit attachment point.

Some proton-carbon correlations, not clearly evident because of partial overlapping of the signals, have been confirmed by INAPT experiments (Fig. 2) that provide long-range heteronuclear connectivity information in a

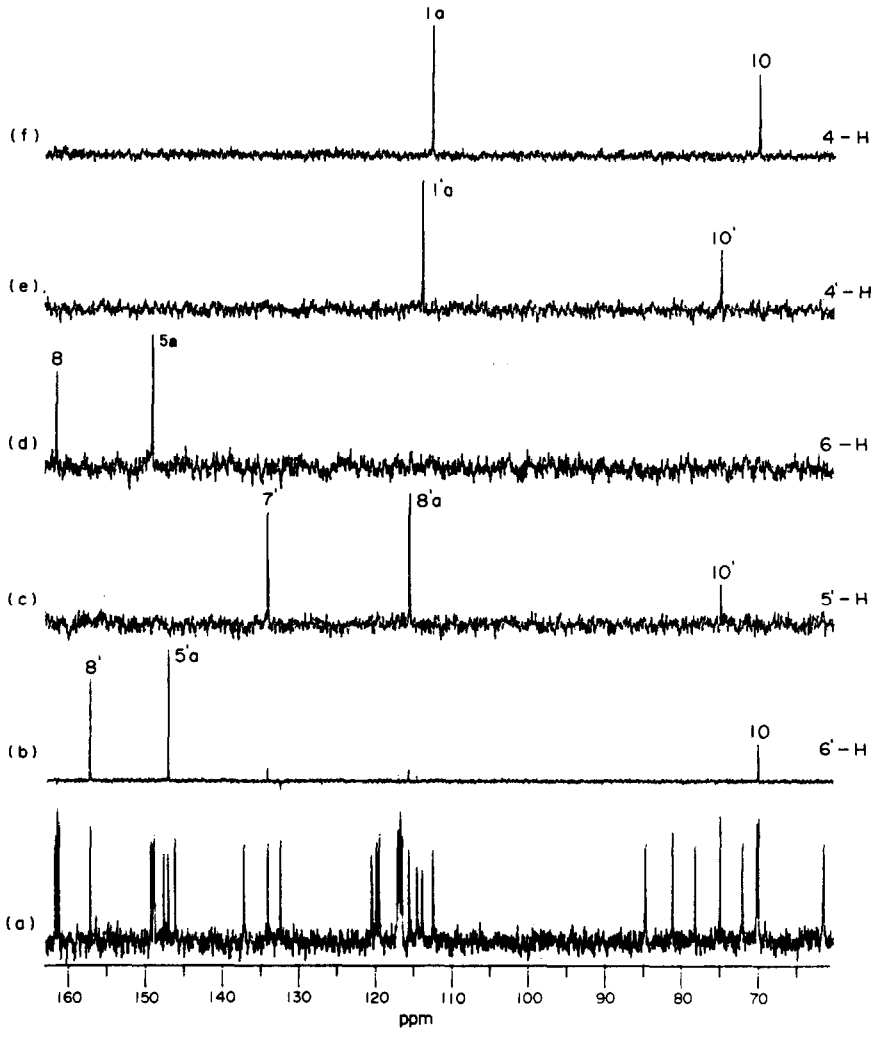


Fig. 2. (a) ^{13}C NMR (75.469 MHz) spectrum of ramnosin **1** in DMSO-d_6 in the δ60–163 region. (b-f) INAPT spectra obtained by positioning the decoupler at the resonances of the protons indicated on the right side of each spectrum. All resonances due to three-bond transfer from the selected proton are assigned in spectra b-f.

highly sensitive way by application of a set of low-intensity radiofrequency pulses to a preselected proton multiplet [8, 9]. In particular, the H-4′, H-5′, H-6′, H-4, and H-5 resonances have been separately irradiated and the corresponding three-bond long-range coupled carbon signals identified.

Compound **1**, to our knowledge, is the first instance of a bianthrone *C*-glycoside. Determination of the structure of other members of this class of natural metabolites from the same source is in progress.

EXPERIMENTAL*

^{1}H NMR spectra were recorded at 400 MHz in DMSO-d_6 on a spectrometer equipped with a dual probe in the Fourier transform mode. One-dimensional spectra were obtained with 5000 Hz spectral width (0–12.5 ppm). The chemical shifts are referred to TMS as int. std. The COSYLR sequence (D_1, 90°, t_1, D_2, 45°, D_2, acquisition) was used to favour cross-peaks relating weakly coupled protons; D_2 was set to 0.08 sec. Each spectrum required a total of 64 pulses in a 512 (f_1) × 2048 (f_2) data matrix size in time domain. The time domain matrix was expanded by zero filling to 1024 points in f_1. Sine bell resolution enhancement was used in both directions.

^{13}C NMR spectra were recorded at 75 MHz at 15 000 Hz spectral width (0–200 ppm). The DEPT [5] expt was performed using a polarization transfer pulse of 135° and a delay adjusted to an average C,H coupling of 160 Hz. One-bond and long-range 2D carbon–proton shift correlation [7] expts were performed with the Bruker XHCORR microprogram using delays D_3 = 3 msec (corresponding to $J_{C,H}$ = 160 Hz) and D_3 = 71 msec (corresponding to $J_{C,H}$ = 7 Hz), respectively. Both expts were recorded using 256 × 1024 [SW = 15 000 Hz (f_2) and 2000 Hz (f_1)] data matrix sizes. Each FID required a total of 480 and 800 scans for one-bond and long-range expts, respectively. In both cases data were processed with exponential multiplication and zero filled. INAPT [8] expts were performed with the Bruker

*With the assistance of Italo Giudicianni.

INEPTRD microprogram using delays $D_2 = D_3 = 36$ msec, corresponding to $J_{C,H} = 7$ Hz.

Droplet counter-current chromatography (DCCC) separations were achieved on a Tokyo Rikakikai model A apparatus, equipped with 300 glass tubes. TLC was carried out on precoated silica gel layers (0.25 mm).

Isolation of **1**. Fr. tubers (5 kg) of *Asphodelus ramosus* L. (collected in the spring in Campania, Italy, and authenticated by the Botanical Garden of the University of Naples) were homogenized in a mechanical stirrer, freeze-dried and extd in a Soxhlet apparatus with petrol (12 hr) and then with Et_2O (12 hr). The Et_2O ext was evapd (3 g) and submitted to DCCC sepn (ascending mode) with $CHCl_3$–MeOH–H_2O (4:4:3, lower phase); flow rate 1 ml/hr. Fractions (30 ml) were collected, then the tubes were discharged (total vol 900 ml) and their contents, on the basis of TLC analysis (silica gel; $CHCl_3$–MeOH, 9:1), collected into six fractions: A (1.8 g), B (120 mg), C (130 mg), D (50 mg), E (80 mg), and F (220 mg) (in increasing order of polarity). Compound **1** was obtained by evapn of fraction F as an amorphous yellow solid, $[\alpha]_D$ −74° (MeOH; *c* 1.1). Elemental analysis: C 64.20%, H 4.89% (calc. for $C_{36}H_{32}O_{13}$: C 64.27%, H 4.80%). Spectral data: see text, Table 1 and Figs.

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CRASSIFOLIOSIDE, A CAFFEIC ACID GLYCOSIDE ESTER FROM *PLANTAGO CRASSIFOLIA*

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Key Word Index *Plantago crassifolia*; Plantaginaceae; caffeic acid glycoside ester; crassifolioside.

Abstract—The structure of crassifolioside, a phenylpropanoid glycoside isolated from *Plantago crassifolia* was shown to be β-(3′,4′-dihydroxyphenyl)-ethyl-(2,3-α-L-dirhamnosyl)-(4-*O*-caffeoyl)-β-D-glucopyranoside.

INTRODUCTION

During recent years, a large number of caffeic acid glycoside esters has been isolated from different plants and from callus cultures [1–6]. Most commonly studied is verbascoside (=acteoside), first isolated in 1963 from *Verbascum sinuatum L.* (Scrophulariaceae) [7], the structure of which was completely elucidated in 1982 [1]. While studying the chemotaxonomy by TLC of the genus *Plantago* [8], we observed in the extracts a new caffeic acid derivative. In this paper we describe the isolation and structure elucidation of this new molecule together with the well known verbascoside using extracts from leaves of *Plantago crassifolia* Forskäl.

RESULTS AND DISCUSSION

Crassifolioside (**1**) was found in leaves and in a higher concentration in roots of *Plantago crassifolia*. The compound was extracted from dried and finely powdered roots and purified as described in Experimental.

It was obtained as an amorphous pale yellow powder, with the elementary composition $C_{35}H_{46}O_{19}$. The FABMS gave, upon addition of Na^+ $[M+Na]^+$ at m/z =793, confirming the M_r as 770. Mild and total acid hydrolysis of **1** were carried out as described by Andary *et al.* [9] to give the hydrolytical products which were in good agreement with the ^1H NMR spectra. The ^1H NMR spectra of compound **1** (Table 1) showed the typical