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MINOR CONSTITUENTS FROM *MAERUA CRASSIFOLIA* FORSSK GROWING IN EGYPT

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تم فى هذا البحث فصل ثلاثة مركبات نـادرة وهـى ٦-ن-ميثيل-٩-بيتـا-د-جلوكوزيد أدينين والذى يفصل لأول مرة من الطبيعة. بالإضافة إلى ٣،٤،٥-تراى ميثوكسى فينول-١-أ-بيتـا-جلوكوبيرانوزيد وكذلك جواياسيل جليسرول. وقد أمكن التعرف على هذه المركبات باستخدام الطـرق الطبيعية والكيميائية والطيفية المختلفة. والمركبان الأخيران يفصلان لأول مرة من العائلة الكبارية.

Three minor components, viz, 6-N-methyl-9-β-D-glucoside adenine [1], 3,4,5,-trimethoxyphenol-1-O-β-D-glucopyranoside [2] and Guaiacyl glycerol [3] were isolated and characterised by chemical and spectral analysis from Maerua crassifolia.

INTRODUCTION

Maerua crassifolia Forssk (F. Capparaceae) is a small tree growing in Egyptian desert.[1,2] The genus *Maerua* has some folkloric uses[3] as ferbifuge, for cephalagia, toothache and infected hairy skin. The alcohol extract of the herb exhibited neuromuscular blocking and antitumor activities.[4,5] Literature dealing with *Maerua*, mentioned little about the constituents of this genus, especially nitrogenous and phenolic compounds.

As a continuation of the study on *M. crassifolia* Forssk[6-9], we have investigated the polar fraction of the methanolic extract of the aerial parts of *M. crassifolia* Forssk, aiming at the isolation of nitrogenous and phenolic compounds.

EXPERIMENTAL

General experimental procedures

Melting points were uncorrected, IR were taken in KBr with Perkin-Elmer (Model 457), ^{1}H- and ^{13}C-NMR spectra were recorded in Bruker AM-400 Spectrometer (at 400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR) using TMS as internal standard. Mass spectra were carried out on Hitachi M-80 spectrometer (Japan). TLC was carried out on silica gel plates (Kieselgel 60 F_{254}, E. Merck), Whatman No.1 sheets were used for PC. For isolation, silica gel (230-400, E. Merck) and reversed phase (RP-18-37, Fuji Gel Hanbai Co. LTD Tokyo, Japan) were used for column chromatography using CIG column system (22 mm i.d χ 30 cm, Kusano Sci, Co., Tokyo, Japan).

The following solvent systems were used:

I-	CH_3OH-H_2O	(1:1)
II-	$CHCl_3$-CH_3OH-H_2O	(70:27:3)
III-	CH_3OH-H_2O	(3:2)
IV-	$CHCl_3$-CH_3OH-H_2O	(80:19:1)

Plant material

The aerial parts of *Maurea crassifolia* Forssk were collected from El-Hafafit in the eastern desert in April 1987. The plant was kindly identified by Prof. Dr. Nabil El-Hadidy, Professor of Taxonomy, Faculty of Science, Cairo University. The aerial parts were air-dried, reduced to No 40 powder and kept in a well-closed container till used.

Extraction

2 kg of the air-dried powdered aerial parts were extracted with methanol by maceration. The methanolic extract (132 g) was mixed with

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500 ml distilled water, then extracted with hexane (5 x 1.5 L.). The hexane fraction (65 gm) was kept for further investigation. The hydromethanolic mother liquor left after evaporation (65 gm) was extracted with $CHCl_3$ (1.5 L. x 5). The hydromethanolic fraction left after extraction of the alcoholic extract with chloroform. This fraction (25 g) was chromatographed on Amberlite (IR-45) column chromatography.

Elution with system I (5 L) afforded compound [1] which was purified by repeated silica gel column chromatography using system II as solvent system.

Fractions eluted with system III (6 L) was evaporated under reduced pressure and rechromatographed over silica gel column chromatography and eluted with system IV where compounds [2] and [3] were obtained.

Acetylation of compound [1]

8 mg of compound [1] was mixed with 10 ml of pyridine and 10 ml of acetic anhydride and left for 24 hours at room temperature. The obtained acetate was purified using CIG column system using silica gel and hexane-ethyl acetate (6:4) as solvent system.

Acid Hydrolysis of [2]

10 mg of [2] was dissolved in 10 mL methanol to which an equal volume of 10% sulphuric acid was added. The mixture was refluxed on a water bath for three hours. The hydrolysate was extracted with chloroform and the mother liquor was evaporated under reduced pressure till dryness and screened for its sugar content using TLC and PC (with authentic sugars). Only one spot having the same R_f as authentic glucose was detected.

Compound [1]

Crystallized as white needles, (methanol), mp 211-213°C, $[\alpha]_D^{25}$ = -51.1° (Methanol). UV λ_{max} (methanol) 264, 263 (sh) and 211 nm,IR (KBr) cm^{-1}, 3450, 1630, 1495, 1400, 1340, 1245, 1101, 1090 and 1058. ^{1}H-NMR (CD_3OD, δ) 3.04 (3H, s, N-CH_3), 3.57-4.05 (6H, m, sugar protons), 5.53 (1H, d, J= 9.3 Hz, H-1´), 8.23 (1H, s, H-8), 8.24 (1H, s, H-2). ^{13}C-NMR (CD_3OD, δ) 28.91 (q, N-CH_3), 62.56 (t, C-6´), 71.12 (d, C-4´), 73.40 (d, C-3´), 78.95 (d, C-2´), 81.18 (d, C-5´), 85.13 (d, C-1´), 121.50 (s, C-5), 140.75 (s, C-4), 140.86 (d, C-8), 154.04 (d, C-2), 157.50 (s, C-6).

EIMS m/z(%): M^+ = 311 corresponding to the molecular formula $C_{12}H_{17}O_5N_5$, other fragments at m/z 192 (11), 178 (89), 150 (96), 149 (100) and 93 (10). SIMS, m/z 312 [M+1] and 334 [M+Na]. FAB/MS m/z 310 [M-1].

Acetate of compound [1]

Gummy material, $[\alpha]_D^{25}$ = -30.0° (chloroform). IR, cm^{-1}, 2950, 1757, 1620, 1520, 1480, 1415, 1365, 1220, 1100, 1055 and 1031. ^{1}H-NMR ($CDCl_3$, δ), 1.80 (3H, s, O= C-CH_3), 2.05 (3H, s, O= C-CH_3), 2.08 (3H, s, O= C-CH_3), 2.09 (3H, s, O=C-CH_3), 2.36 (3H, s, N-CO-CH_3), 3.66 (3H, s, N-CH_3), 4.05 (1H, ddd, J= 9.9, 2.1, 4.8 Hz, H-5´), 4.17 (1H, dd, J= 12.7, 2.1 Hz, H-6´), 4.31 (1H, dd, 12.7, 4.8, H-6´), 5.32 (1H, dd, J= 9.9, 9.6 Hz, H-4´), 5.48 (1H, dd, J= 9.4, 9.6 Hz H-3´), 5.68 (1H, dd, J= 9.4, 9.5 Hz, H-2´), 5.98 (1H, d, J= 9.5 Hz, H-1´), 8.24 (1H, s, H-8) and 8.78 (1H, s, H-2). ^{13}C-NMR ($CDCl_3$, δ) 35.21 (q, O= C-N-CH_3), 61.48 (t, C-6´), 67.75 (d, C-4´), 70.14 (d, C-3´), 72.89 (d, C-2´), 75.22 (d, C-5´), 80.62 (d, C-1´), 125.98 (s, C-5), 140.99 (d, C-8), 152.26 (d, C-2), 153.03 (s, C-4), 154.37 (s, C-6). EIMS, m/z at 521 [M^+], 429, 420, 331 and base peak at 169.

Compound [2]

White needle crystals, (methanol), mp 210-212°C. It is soluble in ethyl acetate, acetone, ethanol and methanol, sparingly soluble in chloroform and insoluble in water. IR (KBr) cm^{-1}, 3300, 1612 and 1515. ^{1}H-NMR (C_5D_5N, δ) 6.79 (2H, s, H-2 & H-5), 5.61 (1H, d, J= 7.4 Hz, H-1´), 4.62 (1H, d, J= 11 Hz, sugar proton), 4.10-4.40 (5H, m, sugar protons), 3.85 (3H, s, 4-OCH_3), 3.73 (6H, s, 3 & 5-OCH_3). ^{13}C-NMR (C_5D_5N, δ) 155.27 (s, C-4), 154.26 (s, C-3 & C-5), 134.54 (s, C-1), 103.00 (d, C-1´), 95.48 (d, C-2 & C-6), 79.17 (d, C-3´ or C-5´), 78.73 (d, C-5´ or C-3´), 75.07 (d, C-

2´), 71.66 (d, C-4´), 62.58 (t, C-6´), 60.68 (q, 4-OCH_3), 55.96 (q, 3- and 5-OCH_3). EIMS m/z at 346 [M^+] corresponding to the molecular formula $C_{15}H_{22}O_9$.

Compound [3]

Colourless amorphous powder (methanol), $[\alpha]_D^{25}$ = -36.2° (methanol), IR, (KBr) cm^{-1}, 3420, 2950, 1625, 1595, 1500, 1405, 1220, and 1190. EIMS m/z at 197 [M^+-OH], 179 [M^+-(H_2O+OH)], 167, 153 (base peak), 137, 125, 122, 110, 106, 93, 78, and 65. ^{1}H-NMR (CD_3OD, δ) 6.99 (1H, d, J= 1.7 Hz, H-2), 6.76 (1H, d, J= 8.1 Hz, H-5), 6.80 (1H, dd, J= 1.7, 8.1 Hz, H-6), 4.51 (1H, d, J= 6.3 Hz, H-7), 3.67 (1H, m, H-8), 3.48 (1H, dd, J= 3.9, 11.3 Hz, H-9a), 3.37 (1H, dd, J= 6.4, 11.3 Hz, H-9b). ^{13}C-NMR (CD_3OD,δ) 147.24 (s, C-1), 111.69 (d, C-2), 148.93 (s, C-3), 134.87 (s, C-4), 115.95 (d, C-5), 120.74 (d, C-6), 75.52 (d, C-7), 77.64 (d, C-8), 64.32 (t, C-9), 56.46 (q, OCH_3).

Acetate of compound [3]

8 mg of [3] was subjected to acetylation as mentioned before. The obtained acetate was purified using CIG column system (adsorbent RP-18, solvent system methanol-water 1:1), $[\alpha]_D^{25}$ = -27.5 (chloroform), IR, (KBr) cm^{-1}, 1745 ($COCH_3$). ^{1}H-NMR ($CDCl_3$, δ), 6.95 (1H, d, as a part of m, H-2), 7.02 (1H, d, J= 8.5 Hz, H-5), 6.95 (1H, dd, as a part of m., H-6), 5.96 (1H, d, J= 7.5 Hz, H-7), 5.42 (1H, m, H-8), 4.28 (1H, dd, J= 3.8, 12.1 Hz, H-9a), 3.82 (1H, dd, J= 5.7, 12.1 H-9b), 3.84 (3H, s, OCH_3), 2.04, 2.06, 2.09 (each 3H, s, alcoholic acetates), 2.30 (3H, s, phenolic acetate). ^{13}C-NMR ($CDCl_3$, δ), 147.24 (s, C-1), 111.47 (d, C-2), 151.48 (s, C-3), 134.77 (s, C-4), 119.85 (d, C-5), 123.19 (d, C-6), 72.32 (d, C-7), 73.53 (d, C-8), 62.20 (t, C-9), 56.12 (q, OCH_3). EIMS m/z: 382 (M^+, $C_{18}H_{22}O_9$), 340, 280, 196, 178, 167, 153 (100%).

RESULTS AND DISCUSSION

The hydromethanolic fraction left after extraction of the alcoholic extract with chloroform, upon repeated column chromatography afforded three compounds [1] (67.8 mg), [2] (112.4 mg) and [3] (13.6 mg).

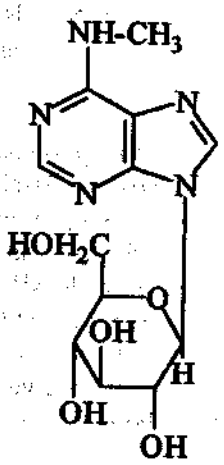
Compound [1]

The UV spectrum showed absorption bands characteristic for adenine nucleoside. ^{1}H-NMR spectrum showed the presence of two protons in the downfield area characteristic for C-2 and C-8 protons in 9-substituted purine nucleus[10] and sugar protons. The ^{13}C-NMR spectrum (CD_3OD) showed the presence of 12 carbon signals, one of them is quartet for N-CH_3 and five for adenine and the other six carbons for hexose sugar.

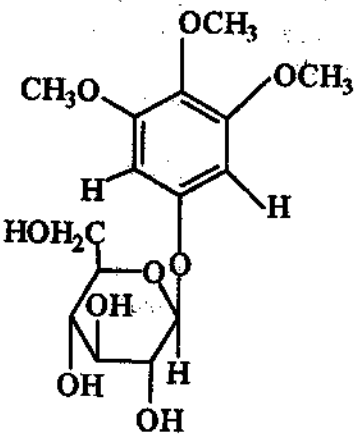
The EIMS, SIMS and FAB/MS data indicated the molecular formula $C_{12}H_{17}O_5N_5$. 0.5 H_2O and the presence of hexose attached to adenine base. Analysis of the acetate gave evidence about the base and the sugar moiety, since the ^{1}H-NMR showed the presence of very clear coupled 7 protons of the hexose. Assignment of the ring and sugar protons and carbons was determined by means of two-dimensional heteronuclear COSY (^{1}H-^{13}C COSY) and ^{1}H-^{13}C Long Range COSY (COLOC) [Correlation spectroscopy via Long range Coupling].

The sugar attachment with the aglycone part was determined by using Long range Selective Proton Decoupling experiments (LSPD)as shown in Fig. 1, since in normal long-range coupled ^{1}H-^{13}C spectrum, C-4 proton appears as multiplet (actually as ddd with protons on C-8, C-2 and C-1´, upon irradiation of the anomeric proton, H-4 appears as dd while the splitting pattern of C-5 proton did not change in this irradiation process. While upon irradiation at C-8 both C-4 and C-5 proton splitting pattern is changed indicating that the sugar moiety is linked to N-9 not N-7. In addition to that, ^{1}H-NMR spectrum showed the presence of 5 acetate signals, four of them are characteristic for the hexose sugar and the fifth is for N-CO-CH_3.

From the coupling constant of the sugar protons, it is clear that the sugar moiety is glucose, the coupling of all the sugar protons is axial coupling, also the coupling constant of the anomeric proton is 9.5 Hz indicating the ß-configuration of the sugar moiety.[11] Since there is no much data about the ^{13}C-NMR spectrum



Compound [1]



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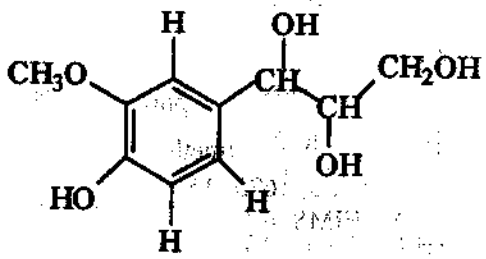

Compound [3]

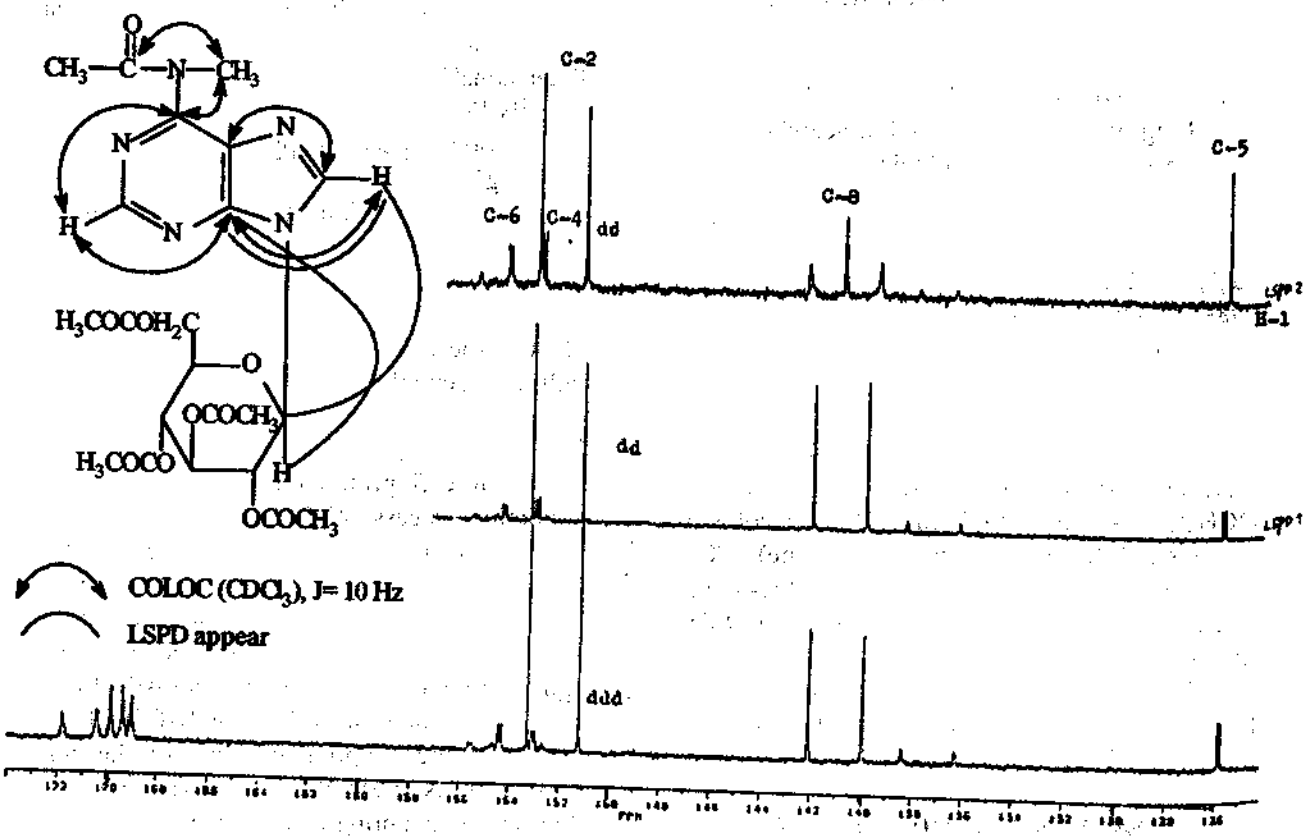

Fig. (1): COLOC and LSPD assignment of Compound [1] acetate.

assignment of the hexose attached to the adenine, the assignment was carried out using ^{1}H-^{13}C COSY, and this estimation was supported by ^{1}H-^{13}C COLOC for the full assignment.

The EIMS,SIMS, FAB/MS (experimental) and elemental analysis data of [1] and EIMS of its acetate support such data. The fragmentation pattern is summarised in Fig. 2. Accordingly compound [1] could be characterised as 6-N-methyl-9-ß-D-glucoside adenine. This is the first report on the isolation of this compound from natural source.

Compound [2]

This compound was obtained as white needle crystals m.p 210-212°C (methanol). It is soluble in ethyl acetate, methanol and acetone, sparingly soluble in chloroform and responded positively to the test for glycosides.

The IR spectrum revealed the presence of hydroxyl groups (3300 cm^{-1}) and aromatic absorption at 1612 & 1615 cm^{-1}.

MS showed M^{+} at m/z 346 corresponding to the formula $C_{15}H_{22}O_9$ and a base peak at m/z 184 originating from the loss of hexose from the molecule. The acid hydrolysis of [2] afforded besides the aglycone, a sugar which was identified as glucose (TLC and PC alongside with reference sugars).

The ^{1}H-NMR of [2] showed a ß-anomeric proton signal at δ 5.61 (1 H, d, J= 7.4 Hz), and the downfield shift of this anomeric proton indicates that the glucose moiety is directly linked to an aromatic residue.

The ^{1}H-NMR of [2], further showed signals for two aromatic protons at δ 6.79 (2 H, s) and three aromatic methoxyls at δ 3.85 (3 H, s) and 3.73 (6 H, s).

The ^{13}C-NMR augumented the previous conclusion showing signals interpreted for tetrasubstituted phenolic ring and three methoxyl signals and further confirmed the -ß-D-glucopyranosidal nature of the glucose moiety present in [2].

Accordingly, [2] was ascribed the structure 3,4,5-trimethoxyphenol-1-O-ß-D-glucopyranoside reported for the first time in Family *Capparaceae*.

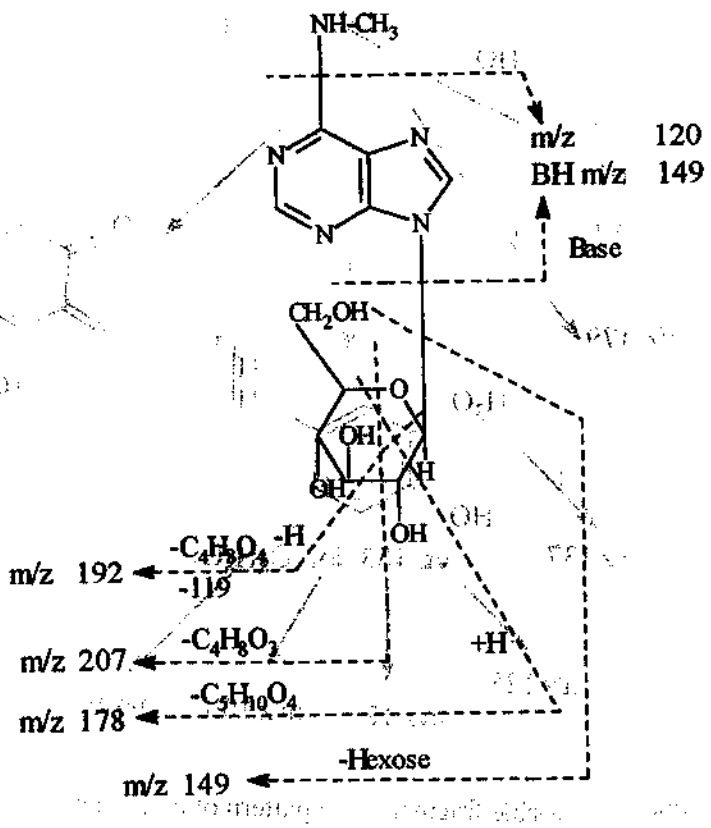

Fig. (2): Possible fragmentation pattern of Compound [1]

Compound [3]

It was obtained as amorphous powder having $[\alpha]_D^{25}$ = -36.2° (Methanol). Its molecular formula was deduced as $C_{10}H_{14}O_5$ "EIMS and ^{13}C-NMR".

The IR spectrum of [3] showed absorption bands assigned for hydroxy and aromatic grouping. The EIMS showed a peak at m/z 197 for M^+ - OH while its acetate revealed a molecular ion peak at m/z 382 with further loss of acetate function to give a peak at m/z 340. Other possible fragments of [3] are shown in Fig 3.

The ^{1}H-NMR of [3] revealed the presence of three aromatic proton signals at δ 6.99 (1 H, d, J= 1.7 Hz), 6.76 (1 H, d, J= 8.1 Hz) and 6.80 (1 H, dd, J= 1.7 and 8.1 Hz). The ^{1}H-NMR further showed one aromatic methoxyl at δ 3.84. In addition, other four protons of glycerol side chain were detected, two of them with geminal coupling for $H_2C.OH$, the multiplet at δ 3.67 for -CH- of $CH(OH)-CH_2(OH)$ in the side chain and the doublet at δ 4.51 (1 H, d, J= 6.3 Hz) was assigned for C-7 proton.

The ^{13}C-NMR of [3] and its acetate showed the presence of trisubstituted phenolic ring, three oxygenated carbon (glycerol) and one methoxyl group. The previous data are in accordance with the structure of guaiacyl glycerol reported here for the first time in Family *Capparaceae*.

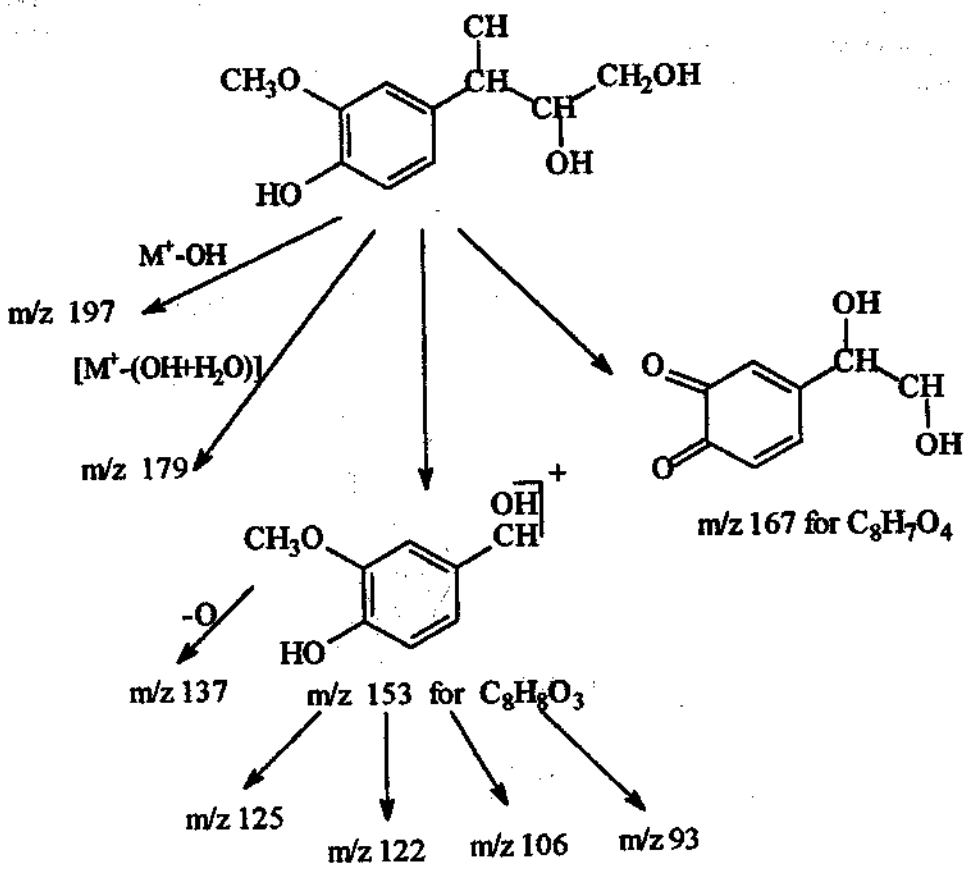

Fig. 3: Possible fragmentation pattern of compound [3]

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