CHAPTER 2

DISCUSSION

Chemical constituents of Garcinia mannii (Guttifereae)

Garcinia is a large genus of evergreen trees or shrubs distributed in tropical Asia, Africa and Polynesia. About thirty species are found in India[1]. The genus Garcinia is known as a source of xanthones, biflavonoids and benzophenones[2-4].

Garcinia mannii is a rain forest tree of west Africa of Cameroon. The twigs and small adventitious roots have been observed to be used as chew-sticks and the dried, powdered root-bark, is reported to be a cure for sever diarrhoea and dysentery [5].

The leaves of Garcinia mannii (2.5 kg) procured from Nigeria, were exhaustively extracted with acetone. The acetone extract was concentrated under reduced pressure. The acetone concentrate was successively extracted with light petroleum ether (60-80 °C), benzene and ethylacetate. The ethylacetate extract gave positive test for flavonoids[6]. On TLC examination, the ethylacetate extract showed four major spots in TEF and BPF solvent systems. Repeated column chromatography over silica gel followed by fractional crystallization and preparative TLC, afforded four crystalline TLC homogenous substances marked as GM-1, GM-2, GM-3 and GM-4.

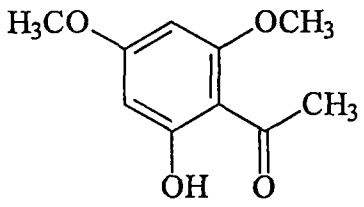
GM-1

GM-1 was obtained from the column by benzene-ethylacetate (9:1) mixture, and was crystallized from benzene-chloroform as white shining crystals (140 mg). m.p. 149 °C. It gave greenish colour with alcoholic solution of ferric chloride. The elemental analysis agreed with molecular formula $C_{10}H_{12}O_4$. The ir spectrum showed the characteristic bands at 1460 cm^{-1} (C=C), 1660 cm^{-1} (C=O) and 3300 cm^{-1} (OH) and uv spectrum gave λ_{max} at 312 nm.

The ^{1}H-nmr spectrum (Table-1), exhibited a sharp singlet integrating for three protons at δ 2.0 assigned to CH_3. Two independent singlets of one proton each at δ 6.38 and δ 7.10 were ascribed to H-3 and H-5 protons respectively. Two singlets at δ 3.16 and δ 3.41 integrating for three protons each, indicated the presence of two methoxyl groups. On the basis of elemental analysis, ir, uv and ^{1}H-nmr spectroscopy, the compound GM-1 was identified as dimethyl ether of phloroacetophenone (I).

The assigned structure was further supported by ^{13}C-nmr spectrum (Table-2) which showed the presence of three oxygenated carbons at 166.2, 165.6 and 163.4, a carbonyl carbon at 180.1 and a methyl group at 16.5 ppm. The mass spectrum showed the molecular ion peak at m/z 196.

In the light of above results, GM-1 was characterized as 2, 4-dimethoxy-6-hydroxy acetophenone (dimethylether of phloroacetophenone)[7](I).



(I)

Table-1

^{1}H-nmr data of GM-1, values on δ- scale

Assignment	No. of protons	Signals
CH_3	3	2.0 (s)
OCH_3	3	3.16 (s)
OCH_3	3	3.41 (s)
H-3	1	6.38 (s)
H-5	1	7.10 (s)
OH	1	12.5 (s)

s= singlet, spectrum run in $CDCl_3$ at 300 MHz, using TMS as internal standard.

Table-2

^{13}C-nmr data of GM-1, values on δ- scale

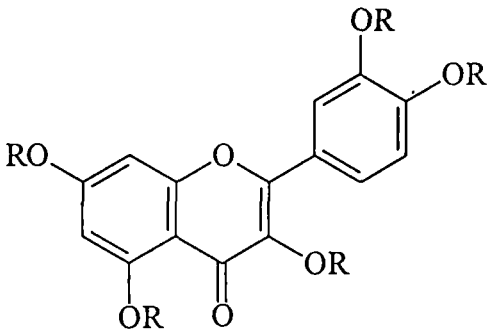
Carbon No.	Assignment
C-1	104.8
C-2	166.2
C-3	91.2
C-4	165.6
C-5	94.1
C-6	163.4
C=O	180.1
CH_3	16.5
OCH_3	55.3
OCH_3	56.1

GM-2

GM-2 was eluted from the column by benzene-ethyl acetate (8:2) mixture. It was crystallized from chloroform-methanol as yellow shining crystals (100 mg), m.p. 311-12 ^{O}C. It was identified as quercetin (IIa) by m.p., m.m.p. and co-chromatography with an authentic sample of quercetin. It gave an acetate m.p. 194-95 ^{O}C and penta methyl ether m.p. 151-52 ^{O}C. Its identity as quercetin was further confirmed by comparing its spectral data with those of an authentic sample of quercetin[8].

The ^{1}H-nmr spectrum of its acetate GM-2 (Ac) (IIb) showed the signals due to five acetoxyl groups in the range of δ 2.35-2.38. The meta-coupled doublets at δ 6.79 (J=2.5 Hz) and δ 7.21 (J=2.5 Hz) were assigned to C-6 and C-8 protons of A-ring respectively. The B-ring protons showed the ABX pattern, two doublets at δ 7.73 (J=2.5 Hz) for H-2` and δ 7.27 (J=9.0 Hz) for H-5` and quartet at δ 7.67 (J_1= 2.5 Hz, J_2=9.0 Hz) for H-6`.

In the light of the above results, the compound GM-2 was assigned the structure as 3, 5, 7, 3`, 4`-pentahydroxy flavone (quercetin) (IIa), supported by the mass spectrum which showed molecular ion peak at m/z 302.

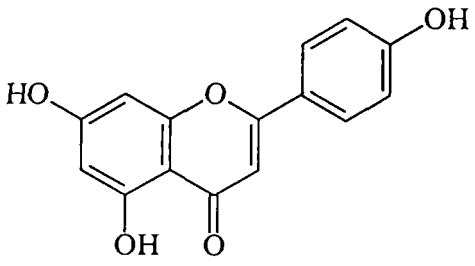


(II)

a)R=H
b)R=Ac

GM-3

GM-3 was eluted from the column by benzene-ethyl acetate (7:3) mixture. It was characterized as apigenin[8] (III), by comparison with an authentic sample (R_f-value, m.p., m.m.p. and co-chromatography). The identity of the compound, GM-3, as apigenin (III, 5, 7, 4`- trihydroxy flavone) was further confirmed by ^{1}H-nmr studies of its acetate, m.p. 183-84 ^{O}C (Table-3).



(III)

Table-3

^{1}H-nmr data of GM-3 (Ac), values on δ- scale

Assignment	No. of protons	Signals
H-2`,6`	2	7.76 (d, J= 8.6 Hz)
H-3`,5`	2	7.20 (d, J= 8.6 Hz)
H-8	1	6.89 (d, J= 2.5 Hz)
H-6	1	6.74 (d, J= 2.4 Hz)
H-3	1	6.58 (s)
OAc-5	3	2.44 (s)
OAc-4`,7	6	2.35 (s)

s= singlet, d= doublet, spectrum run in $CDCl_3$ at 300 MHz, using TMS as internal standard.

GM-4

The fraction obtained by the elution of the column by benzene-ethyalacetate (1:1), on TLC examination was found to be an intricate mixture of several compounds, with one of them as the major one. Repeated column chromatography followed by preparative-TLC failed to separate the mixture. However, by PTLC, most of the minor fractions were removed. The major fraction along with some very minor impurities was analysed by ^{1}H-nmr spectrum. As the compound was not absolutely pure, the spectrum could not be successfully interpretted. However, the presence of OCH_3 group was ruled out by the absence of any peak in the ^{1}H-nmr spectrum.

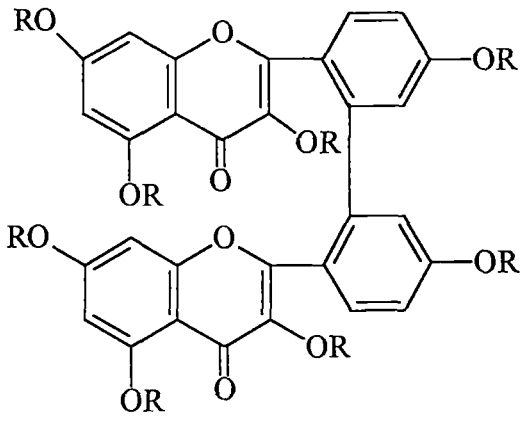
The mixture was methylated with dimethyl sulphate and major band was separated by PTLC. It was purified by crystallization (165 mg), m.p. 189-90 ^oC. The uv spectrum and colour test[6] showed it to be a flavone. Elemental analysis gave the molecular formula as $C_{38}H_{34}O_{12}$, corresponding to the molecular ion peak at m/z 682. The molecular weight indicated it to be a methyl ether of biflavone. The structure of the methyl ether was established by ^{1}H-nmr, ^{13}C-nmr and mass spectroscopy.

The ^{1}H-nmr spectrum of GM-4 (Me) (Fig-1, Table-4), showed the presence of eight methoxyl groups by four independent singlets of six protons each at δ 3.68, 3.85, 3.91 and 3.94 respectively. The A-ring protons appeared as two singlets for two protons each at δ 6.16 ascribed to H-I-6 and H-II-6 and at δ 6.20 attributed to H-I-8 and

H-II-8. The B-ring protons showed typical ABX pattern consisting of an ortho-coupled doublet at δ 7.32 (J= 8.1 Hz) assigned to H-I-6` and H-II-6` and a double doublet for four protons in the range of δ 6.09-6.17 (J_1= 8.0 Hz, J_2= 2.0 Hz) corresponded to H-I-3`, 5` and H-II-3`, 5` protons. Hence, it indicated that H-I-2` and H-II-2` are involved in inter flavonoid linkage which was further proved by the fragment ions at m/z 475, 460, 444, 416, 295, 268 in the mass spectrum (Fig-2, Scheme-1). The RDA fragments appeared at m/z 180 and 502. Other fragments are rationalized from the Scheme-1.

The above assigned structure was also supported by the ^{13}C-nmr spectrum (Fig-3, Table-5) of GM-4 (Me) in which both the carbonyl groups were observed at 188.8 ppm. It also showed a downfield shift for I and II-C-2`, further confirming the inter flavonoid linkage between I-C-2` and II-C-2`.

In view of the above facts GM-4 (Me) was assigned the structure I-3, II-3, I-5, II-5, I-7, II-7, I-4`, II-4`-Octamethoxy [I-2`, II-2`] biflavone (IVb). The parent compound, GM-4, was therefore, assigned the structure as I-3, II-3, I-5, II-5, I-7, II-7, I-4`, II-4`-Octahydroxy [I-2`, II-2`] biflavone (IVa) which is being reported for the first time.



(IV)

a) R=H
b) R=Me

Table-4

^{1}H-nmr data of GM-4 (Me), values on δ- scale

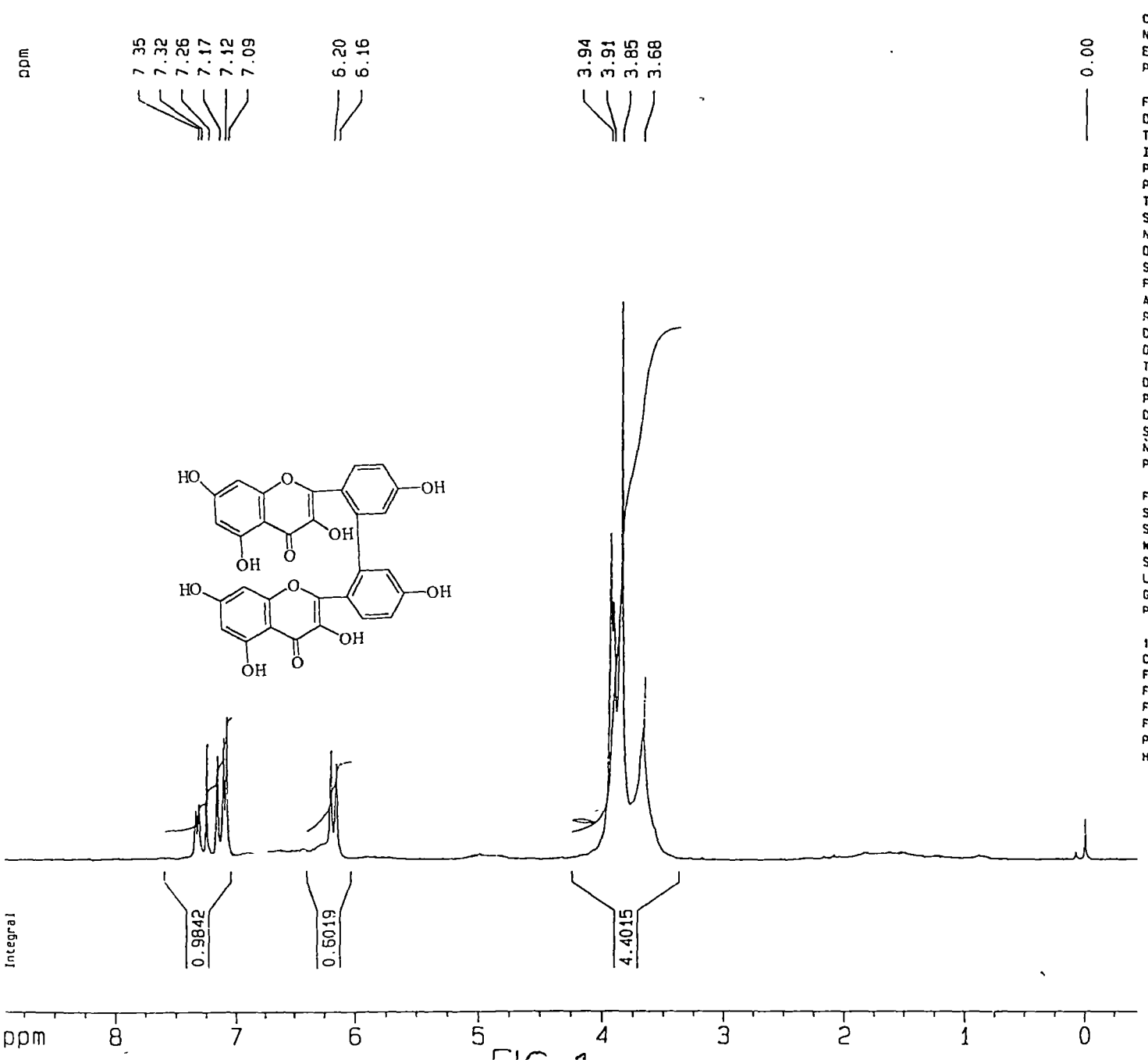
Assignment	No. of protons	Signals
2 x OMe	6	3.68 (s)
2 x OMe	6	3.85 (s)
2 x OMe	6	3.91 (s)
2 x OMe	6	3.94 (s)
H-I-6, H-II-6	2	6.16 (s)
H-I-8, H-II-8	2	6.20 (s)
H-I-3`,5` and H-II-3`, 5`	4	7.09-7.17 (dd, J_1= 8.0 Hz, J_2= 2.0 Hz)
H-I-6`, H-II-6`	2	7.32 (d, J= 8.1 Hz)

s= singlet, d= doublet, dd= double doublet, spectrum run in $CDCl_3$ at 300 MHz, using TMS as internal standard.

Table-5

^{13}C-nmr data of GM-4 (Me), values on δ- scale

Carbon No.	Assignment
8 x OCH_3	55.64
	55.99
	56.10
	56.28
I, II-C-2	160.6
I, II-C-3	105.8
I, II-C-4	188.8
I, II-C-5	159.6
I, II-C-8	98.4
I, II-C-7	162.6
I, II-C-6	98.6
I, II-C-9	151.6
I, II-C-10	108.8
I, II-C-1`	119.9
I, II-C-2`	129.8
I, II-C-3`, 5`	113.4
I, II-C-4`	165.0
I, II-C-6`	128.0

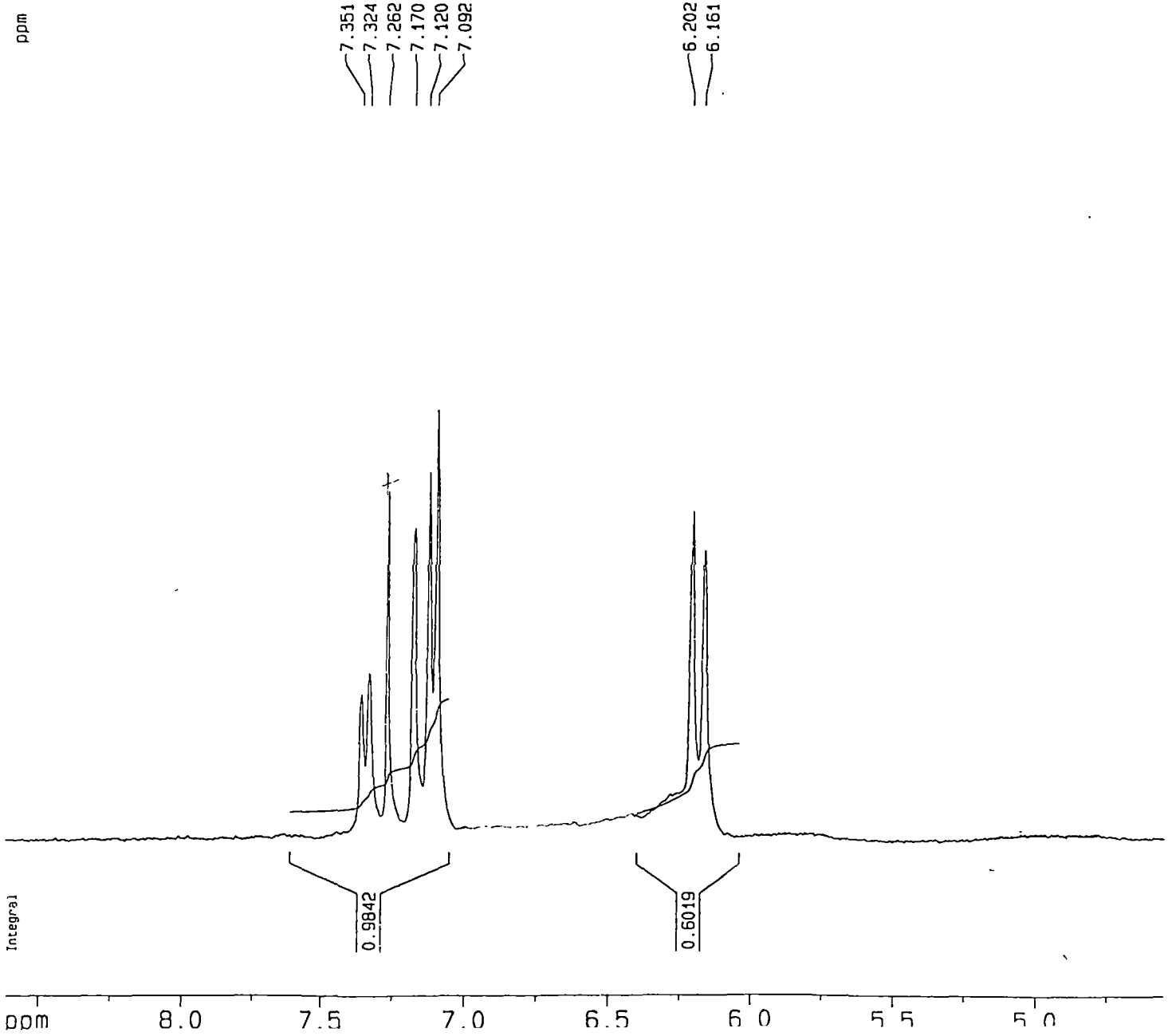


Current Data Parameters
NAME 1a50.rr
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 18.33
INSTRUM drx300
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 32
DS 0
SWH 4789.272 Hz
FIDRES 0.146157 Hz
AQ 3.4210291 sec
RG 256
DW 104.400 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
P1 6.88 usec
DE 6.00 usec
SFO1 300.1319936 MHz
NUC1 1H
PL1 -3.00 dB

F2 - Processing parameters
SI 16384
SF 300.1300056 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 8.931 ppm
F1 2680.52 Hz
F2P -0.439 ppm
F2 -131.73 Hz
PPMCM 0.46850 ppm/cm
HZCM 140.61218 Hz/cm



Current Data Parameters

NAME	1o50.rr
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	500000
Time	18.33
INSTRUM	drx300
PROBHD	5 mm Multinu
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	32
DS	0
SWH	4789.272 Hz
FIDRES	0.146157 Hz
AQ	3.4210291 sec
RG	256
DW	104.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
P1	6.88 usec
DE	6.00 usec
SFO1	300.1319936 MHz
NUC1	1H
PL1	-3.00 dB

F2 - Processing parameters

SI	16384
SF	300.1300056 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.00

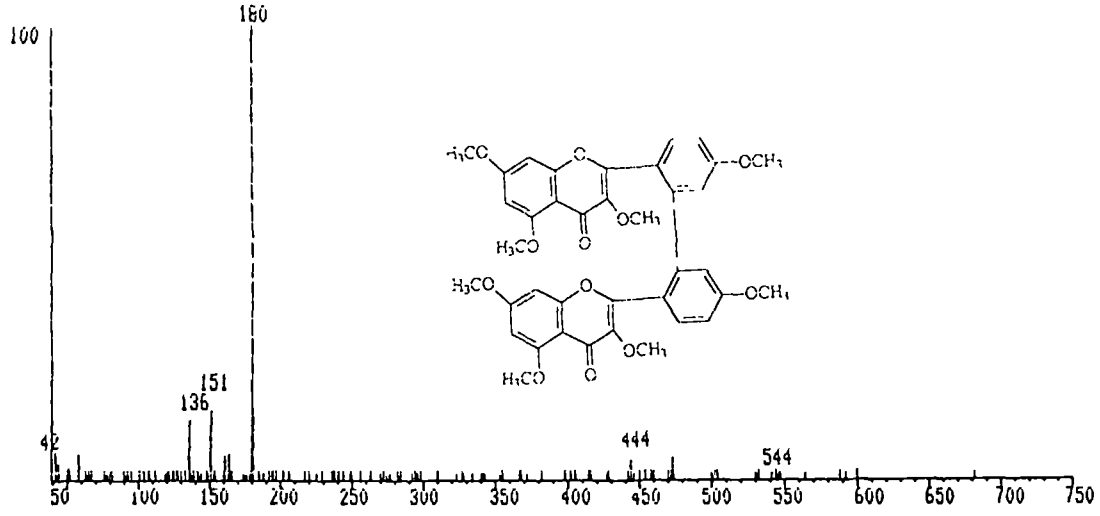
1D NMR plot parameters

CX	20.00 cm
F1P	8.614 ppm
F1	2585.36 Hz
F2P	4.546 ppm
F2	1364.26 Hz
PPMCM	0.20343 ppm/cm
HZCM	61.05529 Hz/cm

केo औo अनुo सo
C. D. R. I.

CENTRAL DRUG RESEARCH INSTITUTE
12-23-1999

Scan : 13 RT= 1:29 No.ions= 140 Base= 37.4%F TIC= 69123



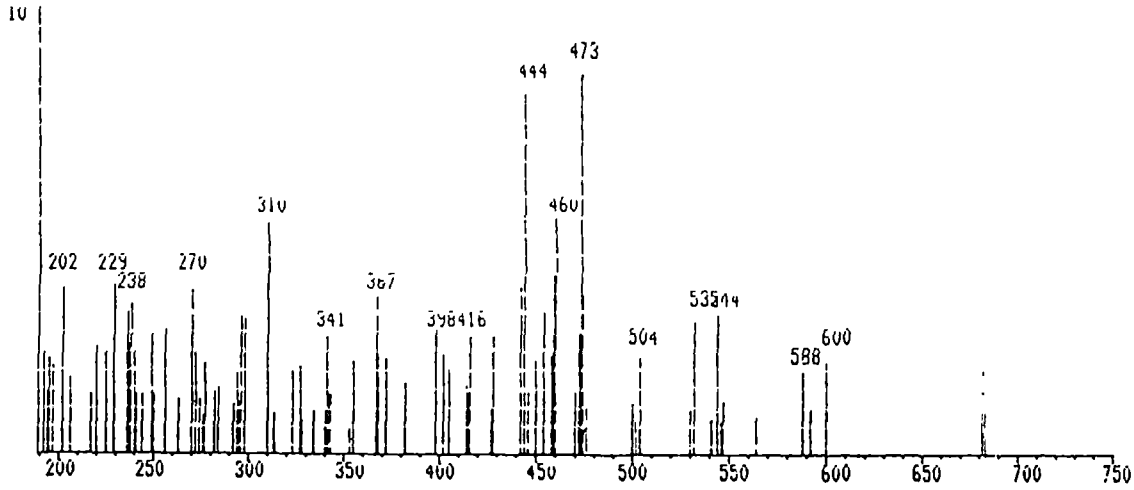
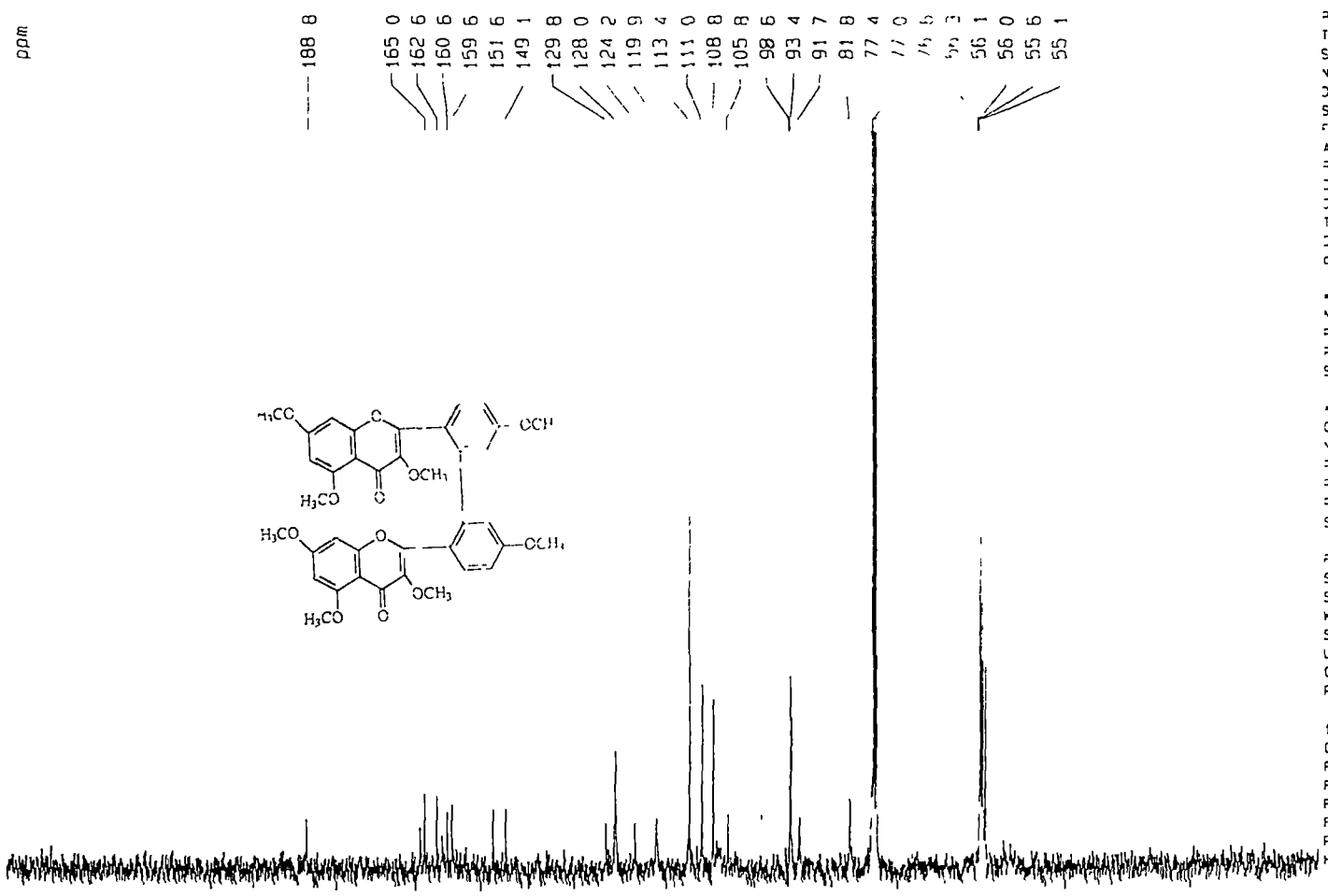


FIG-2



Current Data Parameters
NAME 1a50me4 csic
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20001204
Time 10 23
INSTRUM DRX300
PROBHD 5 mm Multinu
PULPROG zgdc
TD 32768
SOLVENT MeOH CD_3
NS 4741
DS 0
SWH 19960 080 Hz
FIDRES 0 609133 Hz
AQ 0 8208884 sec
RG 11585 2
DW 25 050 usec
DE 6 00 usec
TE 300 0 K
D1 2 00000000 sec
d11 0 03000000 sec

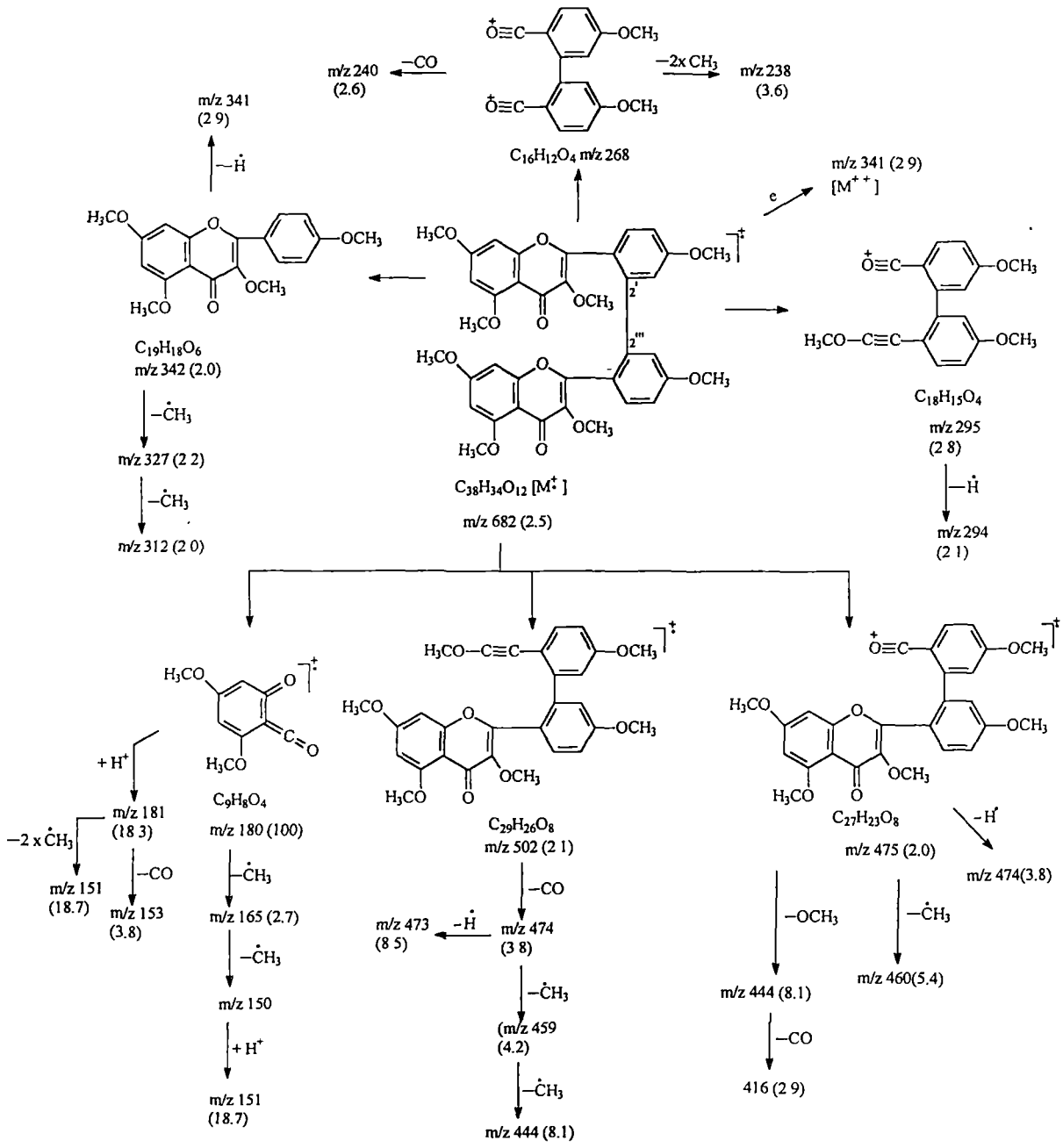
********** CHANNEL f1 ********
NUC1 13C
P1 10 00 usec
PL1 -3 00 dB
SFO1 75 4767751 MHz

********** CHANNEL f2 ********
CPDPRG2 waltz16
NUC2 1H
PCPD2 100 00 usec
PL2 3 00 dB
PL12 20 00 dB
SFO2 300 1319936 MHz

F2 - Processing parameters
SI 16384
SF 75 4677515 MHz
WDW EM
SSB 0
LB 3 00 Hz
GB 0
PC 1 00

1D NMR plot parameters
CX 20 00 cm
F1P 248 750 ppm
F1 18772 61 Hz
F2P -10 837 ppm
F2 -817 84 Hz
PPMCM 12 97935 ppm/cm
HZCM 979 52234 Hz/cm

FIG-3



Scheme I

EXPERIMENTAL

The melting points were taken on a Kofler block and are uncorrected. All **ultraviolet** spectra were measured on Beckmann Model DU and Pye Unicam PU-8800 spectrophotometers in methanol / ethanol. **Infrared** spectra were taken on Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The **mass** and ^{1}**H-nmr** spectra were obtained from different institutes in the country and outside. The **mass** spectra were mostly measured in E.I. mode on Jeol D-300 while, the ^{1}**H-nmr** spectra were usually recorded on Varian EM-360 L (60 MHz), 270 MHz, JEOL 4H-100 MHz, Perkin Elmer R-32 (90 MHz), Brucker dpx 200 MHz, DRX 300 MHz and WM 400 MHz in $CDCl_3$ / DMSO-d_6 using TMS as internal standard.

The silica gel used for different chromatographic purposes, was obtained from E. Merck (India), E. Merck (Germany) and SRL (India). TLC solvent systems used were benzene-pyridine-formic acid (BPF, 36:9:5), toluene-ethylformate-formic acid (TEF, 5:4:1), ethylacetate-ethylmethylketone-acetic acid-water
(EtOAc-EtMeCO-AcOH-H_2O, 5:3:1:1; 20:3:1:1; 30:3:1:1), ethylacetate-methanol-water (EtOAc-MeOH-H_2O, 8:1:1), petrol-benzene (2:8) n-butanol-acetic acid-water (BAW, 4:1:5), n-butanol-water-ethanol (BEW, 60:28.5:16.5).

Alcoholic ferric chloride, iodine vapours and aniline hydrogen phthalate solutions were used as spray reagents for visualization of spots on TLC and on paper chromatograms.

GM-1

GM-1 was obtained from the column by benzene-ethylacetate (9:1) mixture. It was crystallized from benzene-chloroform as white shining crystals (140 mg). m.p.149 °C. It gave greenish colour with alcoholic solution of ferric chloride.

Analysed for $C_{10}H_{12}O_4$.

Calcd. C, 61.22; H, 6.12%

Foud C, 61.19; H, 6.09%

IR ν_{max}^{KBr} cm^{-1}

3300 (OH), 1660 (C=O), 1460 (C=C).

UV λ_{max} (MeOH) nm

312

^{1}H-nmr (300 MHz, $CDCl_3$), values on δ-scale

2.0 (3H, s, CH_3), 3.16 (3H, s, OMe), 3.41 (3H, s, OMe), 6.38 (1H, s, H-3), 7.10 (1H, s, H-5), 12.5 (1H, s, OH).

^{13}C-nmr (300 MHz,$CDCl_3$),values on δ-scale

16.5 (CH_3), 91.2 (C-3), 94.1 (C-5), 104.8 (C-1), 163.4 (C-6), 165.6 (C-4), 166.2 (C-2), 180.1 (C=O), 55.3 (OCH_3), 56.1 (OCH_3).

Mass m/z (rel. Intensity)

196 [M^+] (38.0), 181 [M-CH_3] (15.1), 166 [181-CH_3] (10.0), 168 [M-CO] (20.2), 153 [M-$COCH_3$] (26.0).

GM-2

GM-2 was eluted from the column by benzene-ethyl acetate (8:2) mixture. It was crystallized from chloroform-methanol as yellow shining crystals (100 mg), m.p. 311-12 °C.

Analysed for $C_{15}H_{10}O_7$.

Calcd. C, 59.60; H, 3.31 %

Foud C, 59.64; H, 3.33 %

UV data with shift reagents λ_{max} nm

MeOH	258, 272 sh, 300 sh, 371
NaOAc	258 sh, 275, 390
$NaOAc/H_3BO_3$	263, 301, 389
$AlCl_3$	273, 304 sh, 335, 457
$AlCl_3/HCl$	264, 358, 429
NaOMe	248 sh, 321

Acetylation of GM-2

GM-2 (20 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on a water bath for about two hours. After cooling, the mixture was poured on crushed ice and left over night. The solid obtained was collected, washed with water and dried. On crystallization from ethylacetate, it gave cream coloured crystals (15 mg), m.p.194-95 ^{O}C.

^{1}H-nmr (60 MHz, $CDCl_3$), values on δ-scale

2.38 (3H, s, OAc), 2.35 (12H, m, 4x OAc), 6.79 (1H, d, J=2.5 Hz, H-6), 7.21 (1H, d, J=2.5 Hz, H-8), 7.27 (1H, d, J=9.0 Hz, H-5`), 7.67 (1H, q, $J_1$=2.5 Hz, $J_2$=9.0 Hz, H-6`), 7.73 (1H, d, J=2.5 Hz, H-2`).

Methylation of GM-2

Crystalline GM-2 (25 mg), dry acetone (30 ml), dimethylsulphate (1 ml) and anhydrous potassium carbonate (0.5 g) were refluxed for 24 hours. The reaction mixture was filtered and the inorganic residue washed several times with hot acetone. On distilling off the solvent, a colourless semi-solid mass was left behind. It was washed with hot petroleum ether to remove the excess of dimethyl sulphate. The solid residue on crystallization from methanol-ethylacetate gave colourless needles (20 mg), m.p. 151-52^{O}C.

GM-3

The fraction obtained from the elution of the column with benzene-ethylacetate (7:3) mixture gave a yellow solid mass which was crystallized from benzene-acetone as yellow needles (180 mg), m.p. 352°C.

Analysed for $C_{15}H_{10}O_5$.

Calcd. C, 66.66; H, 3.70 %

Foud C, 66.78, H, 3.74 %

UV data with shift reagents λ_{max} nm

MeOH	266, 298 sh, 339
NaOAc	279, 304, 377
$NaOAc/H_3BO_3$	268, 301 sh, 338
$AlCl_3$	280, 302, 342,392
$AlCl_3/HCl$	280, 301, 342, 391

Acetylation of GM-3

GM-3 (40 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on water bath for about two hours. On usual work-up, the solid obtained was crystallized from chloroform-methanol as colourless needles (35 mg), m.p. 183-84 °C.

^{1}H-nmr (300 MHz, $CDCl_3$), values on δ-scale

2.35 (6H, s, OAc-4`,7), 2.44 (3H, s, OAc-5), 6.58 (1H, s, H-3), 6.74 (1H, d, J=2.4 Hz, H-6), 6.89 (1H, d, J=2.5 Hz, H-8), 7.20 (2H, d, J=8.6 Hz, H-3`,5`), 7.76 (2H, d, J=8.6 Hz, H-2`,6`).

GM-4

The fraction obtained by the elution of the column by benzene-ethylacetate (1:1), on TLC examination was found to be an intricate mixture of several compounds, one of them was the major one. Repeated column chromatography followed by preparative-TLC failed to separate the mixture. However, by PTLC using TEF (5: 4: 1) system as the developing solvent most of the minor fractions were

removed. The mixture was methylated with dimethyl sulphate and major band was separated. It was purified by crystallization (160 mg), m.p.189-90 ^{O}C. The uv spectrum and colour test showed it to be a flavone. Elemental analysis gave the molecular formula as $C_{38}H_{34}O_{12}$. Further confirmed by the molecular ion peak at m/z 682.

Methylation of GM-4

Crystalline GM-4 (150 mg), dry acetone (30 ml), dimethylsulphate (2 ml) and anhydrous potassium carbonate (0.5 g) were refluxed for 24 hours. After usual work-up the solid obtained was crystallized from chloroform-methanol as white crystals (165 mg), m.p.189-90 ^{O}C.

Analysed for $C_{38}H_{34}O_{12}$

Calcd.	C, 66.86; H, 4.98%
Found	C, 66.90; H, 5.01%

IR ν_{max}^{KBr} cm^{-1}

3200-3400 (br, OH), 1680 (C=O), 1600, 1510, 1440

UV data with shift reagent λ_{max} nm

MeOH	258 sh, 268, 294 sh, 323 sh, 368
NaOAc	276, 304, 387
NaOAc/H_3BO_3	277, 304, 388
$AlCl_3$	262 sh, 278, 303 sh, 351, 424
$AlCl_3$/HCl	263 sh, 279, 303 sh, 351, 424
NaOMe	279, 316, 416 (dec.)

^{1}H-nmr (300 MHz, $CDCl_3$), values of δ-scale

3.68 (6H, s, 2 x OMe), 3.85 (6H, s, 2 x OMe), 3.91 (6H, s, 2 x OMe), 3.94 (6H, s, 2 x OMe), 6.16 (2H, s, H-I-6, H-II-6), 6.20 (2H, s, H-I-8, H-II-8), 7.09-7.17 (4H, dd, J_1=8.0 Hz, J_2=2.0 Hz, H-I-3`,5`, H-II-3`, 5`), 7.32 (2H, d, J=8.1 Hz, H-I-6`, H-II-6`).

^{13}C-nmr (300 MHz, $CDCl_3$), values of δ-scale

55.64, 55.99, 56.10, 56.28 (8 x OMe), 160.6 (I-II-C-2), 105.8 (I-II-C-3), 188.8 (I-II-C-4), 159.6 (I-II-C-5), 98.4 (I-II-C-8), 162.6, (I-II-C-7). 98.6 (I-II-C-6), 151.6 (I-II-C-9), 108.8 (I-II-C-10), 119.9 (I-II-C-1`), 129.8 (I-II-C-2`), 113.4 (I-II-C-3`,5`), 165.0 (I-II-C-4`), 128.0 (I-II-C-6`).

Mass m/z (rel. int.)

682 [$M^{+\cdot}$] (2.5), 502 (2.1), 475 (2.0), 474 (3.8), 473 (8.5), 460 (5.4), 459 (4.2), 444 (8.1), 416 (2.9), 342 (2.0), 341 (2.9), 327 (2.2), 312 (2.0), 295 (2.8), 268, 240 (2.6), 238 (3.6), 181 (18.3), 180 (100), 165 (2.7), 153 (3.8), 151 (18.7), 150 (2.6).

REFERENCES

1. **"The Wealth of India"**, Raw Materials, CSIR, New-Delhi, Vol. IV, P. 99 (1956).
2. C. M. A. da M. Rezende and O. R. Gottlieb, **Biochem. Syst. Ecol.**, **1**, 111 (1973).
3. H. D. Locksley, **'Forschr. Chem. Org. Naturst.'**, **30**, 207 (1973).
4. A. V. Rama rao, G. Venkatswamy and A. D. Pendse, **Tetrahedron Letters**, **1975** (1980).
5. I. R. Irvin, **"Woody Plants of Ghana"**, Oxford University Press, London, P. 145 (1961).
6. J. Shionda, **J. Chem. Pharm. Soc. Japan**, **48**, 214 (1928).
7. H. Wagner and V M. Chari, **Tetrahedron Letters**, **21**, 1799-1802 (1976).
8. V. M. Chari, R. J. Grayer-Barkmerjer, J. B. Harborne and G. Osterdahi, **Phytochemistry**,**20**, 1977 (1981).
9. J. B. Harborne,**"Comparative Biochemistry of the Flavonoids"**, Academic Press, London (1967).