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BIFLAVONE FROM *MANGIFERA INDICA*

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ABSTRACT

The phenolic extractive of the bark of *Mangifera indica* yielded a known biflavone which was characterized by spectral and chemical methods as amentoflawne. This is the first report for the isolation of biflavone from *Mangifera indica.*

Introduction

Mangifera indica L. (Nasir, 1983) belongs to the family Anacardiaceae. Earlier investigations on the various parts of this plant led to the isolation of alkyl gallates, amino acids, sugars, triterpenes, chromones and saponins (Khan, 1988, 1989, 1991, 1992). In view of our continued interest in the chemical constituents of this plant, investigations on the bark of *Mangifera indica* were undertaken. As a result of this study a biflavone, amentoflavone, has been isolated from the bark of this plant. The structure identification of amentoflavone was carried out on the basis of chemical and spectroscopic studies.

Materials and Methods

Plant material and extraction

Dried bark (5 Kg) of *Mangifera indica* was crushed to a coarse powder and exhausted with pet. ether (40-60°C). The combined extracts were concentrated under reduced pressure to give an oily residue. The pet ether exhausted bark was refluxed with Me2CO and the Me2CO extract was concentrated under reduced pressure to afford a gummy mass. It was treated with boiling water. The insoluble brown gummy mass was removed by filtration, dried and rained with EtOAc for 12 hrs. The EtOAc extract was evaporated under reduced pressure to give a dark brown solid (8.1 g). It gave a positive test for the biflavones with zinc-hydrochloric acid.

Column chromatography

A well stirred suspension of silica gel (21)0 g) in dry pet. ether (40-60°C) was poured into a column (200 an x 50 mm). When the adsorbent was well stirred, the excess pet. ether was allowed to pass through the column. The dark brown solid (7A g) adsorbed on silica gel (Merck 7733) was added to column. The column was eluted

with organic solvents successively in increasing order of polarity using pet. ether (40-60°C), Bz , CHCl3 , Bz : EtOAc (1:1, v/v), EtOAc and Me_2CO. Fractions obtained from Bz : EtOAc , EtOAc and Me_2CO gave positive test for biflavonoids and were found to be impure on thin layer chromatography (TLC). These fractions were, therefore, combined and concentrated on a rotary evaporator to give brown residue (3.7 g).

Preparative layer chromatography

Residue (3.7 g) obtained from column chromatography was dissolved in Py and applied to TLC plates (20[20 cm., 0.25 mm thick silica gel). These plates were run in a solvent system, Bz-Py-HCOOH (35:10:5, v/v). The position of bands was marked in UV light. The marked pigment zones were scraped and eluted with dry Me_2CO. The eluate in each case was distilled off to give an oily liquid which on addition to water yielded a yellow precipitate. It was filtered, washed with H_2O and dried. The first fraction thus obtained gave a compound (1.1 g) designated as (A).

Methylation of compound (A)

Compound (A), 100 mg, anhydous K_2CO_3 (4 g), $(CH_3)_2SO_4$ (1 ml) and dry Me_2CO (400 ml) were refluxed on a water bath for 10 hrs. A small portion of the reaction mixture was taken out in a test tube and tested for alc. $FeCl_3$ reaction. Refluxing was continued until it gave a negative alc. FeCl3 test. It was worked up in a usual manner to give a yellow compound (55 mg) identified as amentoflavone hexamethyl ether, m.p. 216-218° (lit. m.p. 222-225°, [Pelter,19701).

EIMS 622(100)$[C_{36}H_{30}O_{10}]^+$ (M^+), 621(31)$[C_{36}H_{29}O_{10}]^+$, 607(33) $[C_{35}H_{27}O_{10}]^+$ (M^+-Me), 592(8) $[C_{34}H_{24}O_{10}]^+$ (M^+ -2 x Me), 312(2)$[C_{18}H_{16}O_5]^+$, 311(5)$[C_{18}H_{15}O_5]^+$, 245(5), 1801(3)$[C_9H_8O]^+$, 135(16) and 132(3)$[C_9H_8O]^+$

IR υ_{mex} (KBr): 3082, 3000, 2945, 2852, 1648(C=0), 1572, 1505, 1495, 1460, 1420, 1385, 1338, 1298, 1213, 1175, 1150.

UV (EtOH) λ_{max}: 226, 288, 311 nm.

Acetylation of (A)

Compound (A) (40 mg) was acetylated with Py (1 ml) and $(CH_3CO)_2O$ (2ml) on a water bath for 3 hrs. It was worked up in a usual manner to give a pure amentoflavone hexaacetate (27 mg), m.p. 220-222^o (lit., m.p. 222-225^o, [Petler, 1970]).

Results and Discussion

The phenolic extractive of the coarsely powdered bark of *Mangffera indica* on purification by solvent fraction and column chromatography followed by TLC revealed different bands. The usual colour reactions and UV spectra in ethanol indicated them to be biflavones. They were separated by preparative layer chromatography using Bz-Py-HCOOH (35:10:5, v/v) as the developing solvent system. The first band on methylation and TLC examination was found to be a pure methyl ether. It was identified as hexa-*O*-methyl amentoflavone by comparison of Rf values, characteristic fluorescence in UV light and 'H-NMR spectral data of its acetate and methyl ether. The results of ^{1}H-NMR studies of the methyl ether and acetate of compound (A) are shown in Table 1.

Table 1
Chemical shifts of protons of hexamethyl ether and hexacetate of compound (A)

assignment	chemical shifts (δ) hexamethyl ether	chemical shifts (δ) hexaacetate
H-I-8	6.47 (1H,d,J = 3Hz)	7.26(1H,d,J = 3Hz)
H-I-6	6.32 (1H,d,J = 3Hz)	6.84(1H,d,J = 3Hz)
H-II-6	6.64 (1H,s)	7.01(1H,s)
H-I-3	6.51 (1H,s)	7.98(1H,s)
H-II-3	6.57 (1H,s)	6.57(1H,s)
H-I-6'	7.91 (1H,d,J_1 = 9Hz,J_2 = 3Hz)	7.98(1H,q,J_1 = 8Hz,J_2 = 3Hz)
H-I-2'	7.85 (1H,d,J = 3Hz)	8.03(1H,d,J = 3Hz)
H-I-5'	7.11 (1H,d,J = 9 Hz)	7.48(1H,d,J = 9Hz)
H-II-2',6'	7.37 (2H,d,J = 9Hz)	7.49(2H,d,J = 9Hz)
H-II-3',5'	6.74 (2H,d,J = 9Hz)	7.06(2H,d,J = 9Hz)
OMe-II-5	4.56 (3H,s)	-
OMe-I-5	3.92 (3H,s)	-
OMe-I-7	3.90 (3H,s)	-
OMe-II-7'	3.81 (3H,s)	-
OMe-I-4	3.78 (3H,s)	-
OMe-I-4'	3.76 (3H,s)	-
OAc-I-4	-	2.28 (3H,s)
OAc-II-4'	-	2.23 (3H,s)
OAc-I-7	-	2.05 (3H,s)
OAc-II-7	-	2.01 (3H,s)
OMe-I-5	-	2.45 (3H,s)
OMe-H-5'	-	2.41 (3H,s)

$CDCl_3$, 100 MHz, TMS internal standard.

The ^{1}H-NMR spectrum of methyl ether of compound A indicated that rings I-B and II-B are substituted at position I-3, 4 and 11-4 respectively. Thus rings I-B and II-A of billavone seemed to be involved in interflavonoid linkage. In particular the values showed that 1-3 is linked to either 11-6 or II-8. The observation that in biflavones having aromatic the substituents at I-8 the 1-5, OMe group generally appeared above 4.0 (Table 2), led to believe that the substituent in methyl ether was located at 11-8 and not at 11-6. Further all methoxy groups on change of solvent from deuterochloroform to deuterobenzene moved upfield as in cupressuflavone hexamethyl ether showing that every methoxy group had at least one ortho proton, therefore, a 11-8 rather than all-6 linkage was confirmed.

Table 2

Biflavones	I-5-OMe δ	II-5-OMe δ
Cupressuflavone hexamethyl ether	4.12	4.12
Amentoflavone hexamethyl ether	3.87	4.06
Agathisflavone hexamethyl ether	3.59	4.05
Hinokiflavone pentamethyl ether	4.00	4.80
Compound (A)	3.92	4.56

Acetylation of compound (A) with pyridine and acetic anhydride gave an acetate, m.p. 220-222°C. The ^{1}H-NMR spectrum of the acetate showed six acetoxy groups integrated for 18 protons. The assignment of various protons of acetate is shown in Table 1. Thus compound (A) was concluded to have the structure shown in Fig. 1.

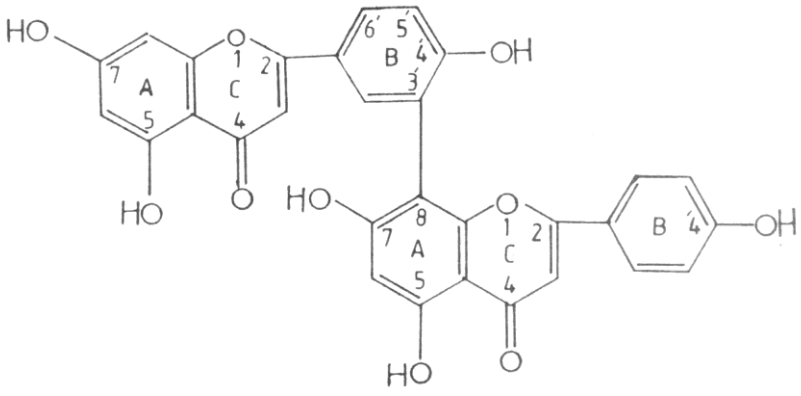

Figure 1. (I-4',II4',I-5,II-5,I-7, II-7-hexahydroxy-[I-3',II-8]-biflavone)

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