CHAPTER IV

DISCUSSION

1. Structure of the New Naturally Occurring Dihydroflavonols

The two new naturally occurring dihydroflavonols characterised as (2R : 3R)-dihydroquercetin-4'-methyl ether and (2R : 3R)-dihydroquercetin-4'-7-dimethyl ether had their structures elucidated from the CD, ultraviolet, infrared, nuclear magnetic resonance and mass spectra.

The arguments for the elucidation of the structure of PS-1 characterised as (2R : 3R)-dihydroquercetin-4'-methyl ether are as follows:-

Colour reactions, ferric chloride and shinoda test indicated that PS-1 was a polyhydroxy flavonoid. The proton NMR data of isolate PS-1 strongly suggested that it was a dihydroflavonol derivative, and the characteristic 11 Hz doublets at 4.48 and 5.03 ppm indicated a *trans*-diaxial arrangement of 2-H and 3-H in such a system. That the signal for 3-H was further split, and on addition of D_2O sharpened to a doublet, indicate that the hydroxyl at C(3) was not methylated. The AB system centered at 5.87 ppm, showing *meta* coupling of approximately 1 Hz, suggested that they were protons at C(6) and C(8) in a dihydroflavonol oxygenated at C(5) and C(7) (Mabry, Markham and Thomas, 1970).

The pronounced bathochromic shift of the band II absorption in the ultraviolet spectrum of PS-1 on addition of sodium acetate indicated

the presence of a free hydroxyl at C(7). Addition of aluminium chloride reagent produced bathochromic shifts of band I (54 nm) and band II (25 nm) characteristic of second free hydroxyl at C(5) (Mabry, Markham and Thomas, 1970). Mass spectral peaks at m/e 166 and 137 substantiated that the remaining hydroxyl and the methoxyl group were indeed on the B-ring (Rodriquez et al, 1972; Harborne, Mabry and Mabry, 1975).

The multiplet at 6.91 (DMSO) or 7.00-7.10 (acetone) in NMR spectrum of PS-I are suggestive of oxygenation at C(3') and C(4') (Mabry, Markham and Thomas, 1970). However, definitive assignment of the substitution pattern could not be made on the basis of these spectra. A portion of the isolate was oxidised using 2N H_2SO_4/air (Pavanasasivam and Sultanbawa, 1975), to the corresponding flavonol, which should be either tamarixetin (Figure XVI a p.105) or isorhamnetin (Figure XVI b). Comparison of the nmr spectrum of the TMS ether of this unknown flavonol with published spectra of the two reference compounds showed that the unknown flavonol corresponded to tamarixetin. This was further substantiated by the stability of the ultraviolet absorption spectrum of the oxidation product in the presence of sodium methoxide. Flavonols having a free hydroxyl at C(4') are unstable in sodium methoxide solution, and their ultraviolet absorption spectra degenerate in a few minutes (Mabry, Markham and Thomas, 1970). The flavonol produced by the oxidation of PS-1 was seen to be stable in the presence of sodium methoxide for at least 24 hours. Also, thin layer chromatography comparison of the oxidation product with isorhamnetin showed them to be different in several systems. A sample of tamarixetin was unfortunately not available.

The absolute stereochemistry at C(2) and C(3) was determined by means of circular dichroism. Comparison of the CD curves of PS-1 with those of dihydroflavonols of known configuration (Gaffield, 1970) established the absolute configuration as 2R, 3R, the signs of the Cotton effects from 400 to 200 nm being respectively +,-,+,+. Thus PS-1 is assigned the structure (2R : 3R)-dihydroquercetin-4'-methyl ether or (2R : 3R)-dihydrotamarixetin).
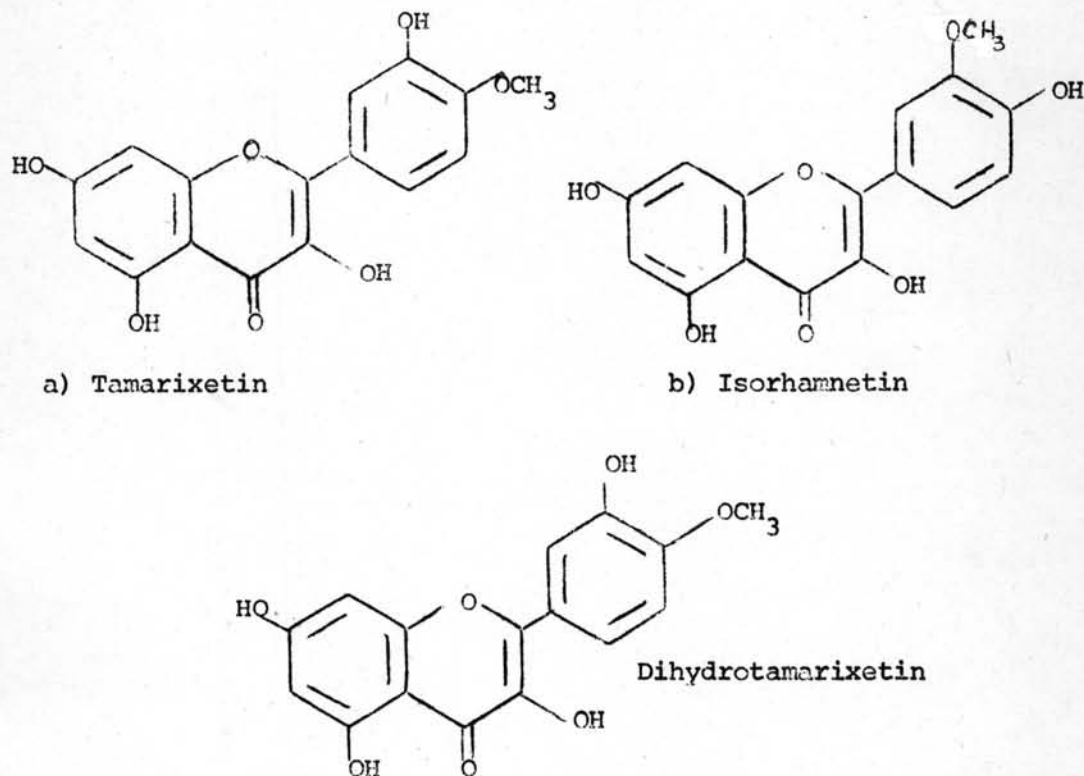
a) Tamarixetin

b) Isorhamnetin

Dihydrotamarixetin

Figure XVI

From mass spectrum, the fragmentation of (2R : 3R)-dihydro-quercetin-4'-methyl ether is proposed corresponding to spectrum peaks as pathway I, Retro-Diels-Alder Cleavage (RDA) and pathway II as follow;

165 (26%)

137 (39%)

(M-29), 289 (44 %)

Pathway II

$\overset{+.}{M}$ 318 (37 %)

Pathway I. (Retro-Diels-Alder Cleavage)

$\left[A_1 + H\right]^+$, 153 (100 %)

$A_1^{+.}$, 152 (7 %)

$B_3^{+.}$, 166 (50 %)

-HCO·

$(M-29)^+$, B_4^+ , 137 (39 %)

The arguments for the elucidation of the structure of PS-2 characterised (2R : 3R)-dihydroquercetin-4', 7-dimethyl ether are as follow :-

Colour reactions, ferric chloride and Shinoda test indicated that PS-1 was a polyhydroxy flavonoid. The NMR spectrum of the isolate PS-2 was identical to that of PS-1 except that it showed the loss of one exchangeable proton and the addition of a second aromatic methoxyl group. Since the ultraviolet absorption spectrum was unchanged on addition of sodium acetate this methoxyl group is located at C(7) (Mabry, Markham and Thomas, 1970). Mass spectral evidence confirmed that the additional 14 mass units were indeed in the A-ring. Sulphuric acid oxidation of PS-2 was used to established the B-ring substitution pattern as in PS-2 rather than PS-2a. Thus, the ultraviolet absorption spectrum of the oxidation product PS-2b was found to be quite stable in sodium methoxide solution, and thin layer chromatography comparison with a reference sample of rhamnazin showed PS-2b to be different in several systems. Circular dichroism again established the absolute configuration to be 2R : 3R. Thus, PS-2 is assigned the structure (2R : 3R)-dihydroquercetin-4', 7-dimethyl ether.
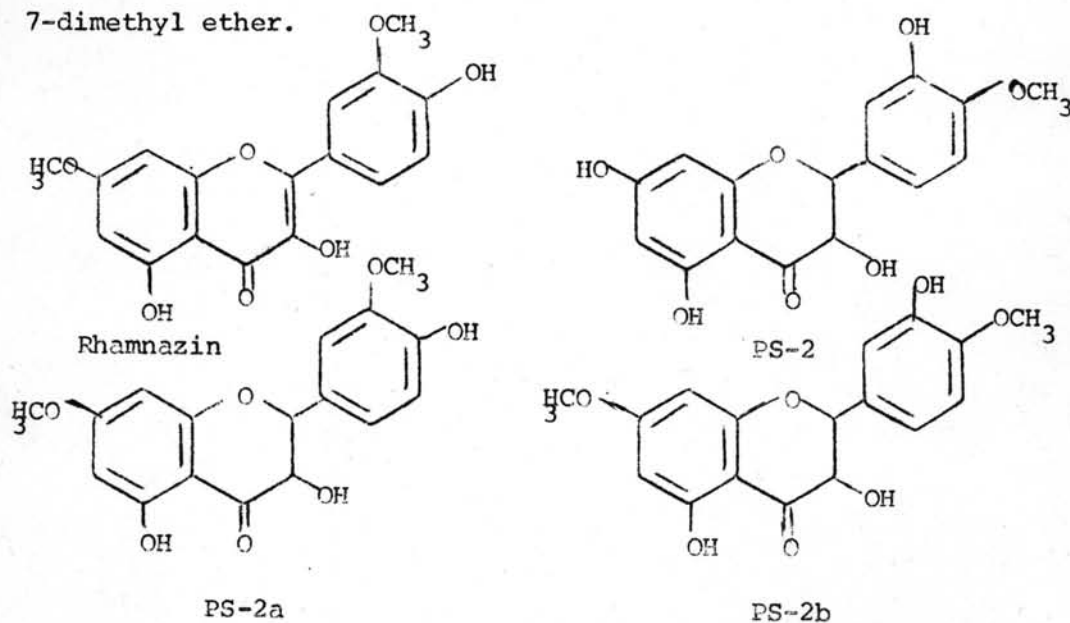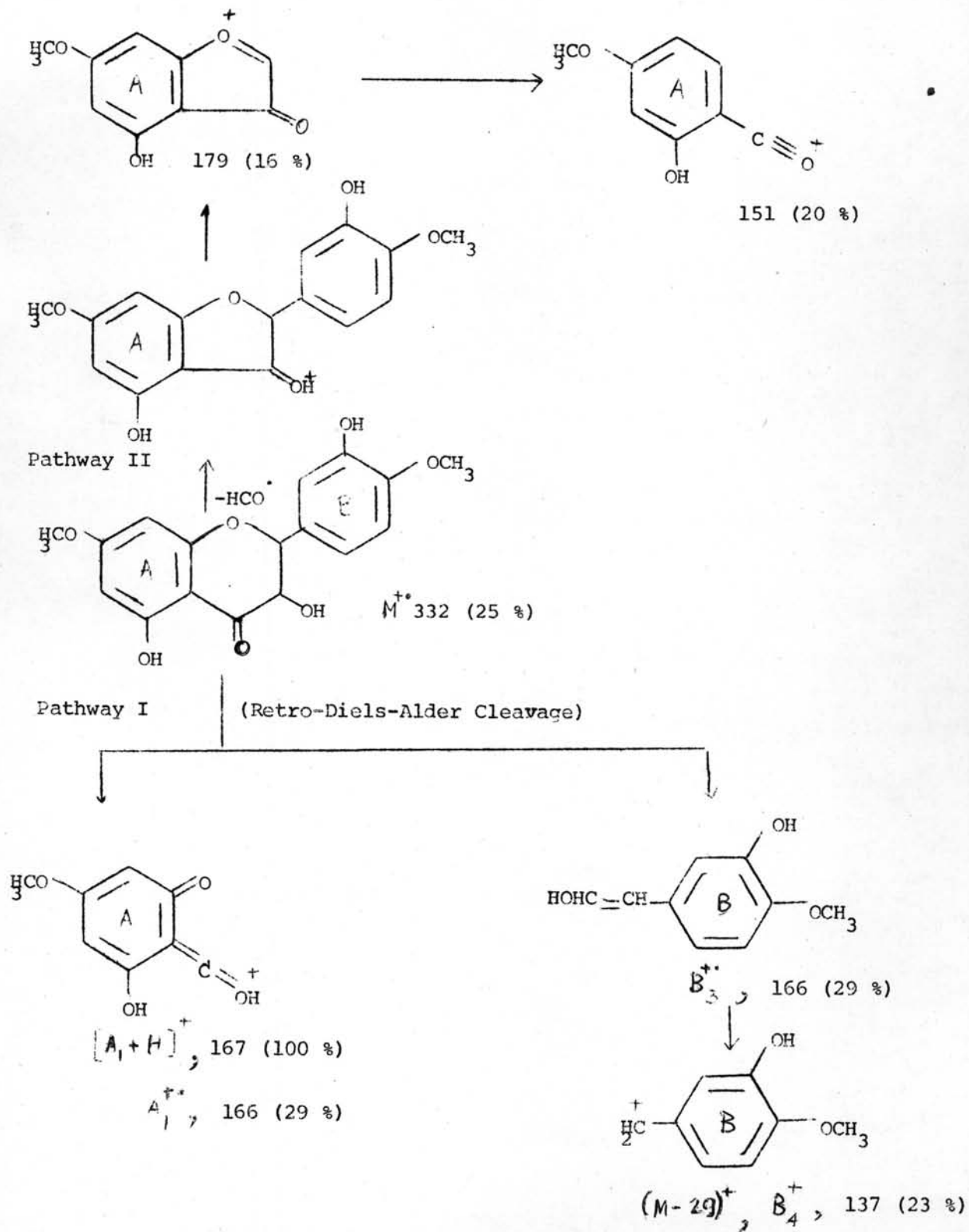

Rhamnazin

PS-2

PS-2a

PS-2b

Figure XVII

From mass spectrum, the fragmentation of (2R : 3R)-dihydro-
quercetin-4', 7-dimethyl ether is proposed corresponding to spectrum
peaks as follow :-

179 (16 %)

151 (20 %)

Pathway II

−HCO˙

$M^{+\bullet}$ 332 (25 %)

Pathway I   (Retro-Diels-Alder Cleavage)

$[A_1 + H]^+$, 167 (100 %)

$A_1^{+\bullet}$, 166 (29 %)

$B_3^{+\bullet}$, 166 (29 %)

$(M-29)^+$, B_4^+, 137 (23 %)

2. The Chemotaxonomic Significance of Dihydroflavonols from *Blumea*
   balsmiferaa DC.

   In most cases, the flavonoid profile has been studied only
superficially. Much of the data available has arisen accidentally as
a by-product of investigations quite unrelated to plant taxonomy.
Dihydroflavonols in Compositae were reported in ten species and three
of them are in tribe Inuleae. *Tessaria dodoneifolia* (Hook, et Arn)
Cabr. (tribe Inuleae) was reported to have dihydroquercetin 3-acetate
(Karka *et al*, 1977). *Jasiania tuberosa* DC. (tribe Inuleae) was reported
to have dihydroquercetin 3',7-dimathyl ether (Gonzale *et al*, 1977) which
was related to dihydroquercetin-4'-methyl ether and dihydroquercetin
$4'$-7-dimethyl ether from *Blumea balsamifera* DC. in this present investi-
gation. Other species which contain dihydroflavonols relating to ones
from *Blumea balsamifera* DC. are *Eupatorium* hybrid, tribe Eupatorieae
and *Artemisia pygmaea* A. Gray, tribe Anthemideae. In 1972, Herz *et al*,
reported the isolation of a dihydroflavonol from *Eupatorium* hybrid which
might have either PS-2 or PS-2a (Fig. XVII, p.107) as its structure.
They were unable to completely define their isolate, having lost their
remaining sample in an unsuccessful oxidation reaction. Comparison of
the aromatic region of the nuclear magnetic resonance spectrum (in DMSO)
of their isolate with that of PS-2 showed them clearly to be different.
Therefore, from the data presented in the reference, Herz *et al*. 1972
and comparison with this isolate PS-2, the structure of the *Eupatorium*
isolate can be defined as 2R : 3R-dihydroquercetin-3',7-dimethyl ether
(PS-2a), which was also reported in *Artemisia pygmaea* A: Gray (Rodriguez
et al, 1972) and *Jasiania tuberosa* DC. (Gonzale *et al*, 1977).

Dihydroflavonols in other species and tribes are unrelated to ones found in tribe Inuleae, Anthemideae and Eupatorieae.

Owing to limited informations available there is no specific dihydroflavonols in tribe Inuleae or even in Compositae. More investigations concerning dihydroflavonols in Compositae, especially in Inuleae are needed to conclude their significance in chemotaxonomy.