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Mass Spectrometry of Derivatives of a Natural Trileucofisetinidin

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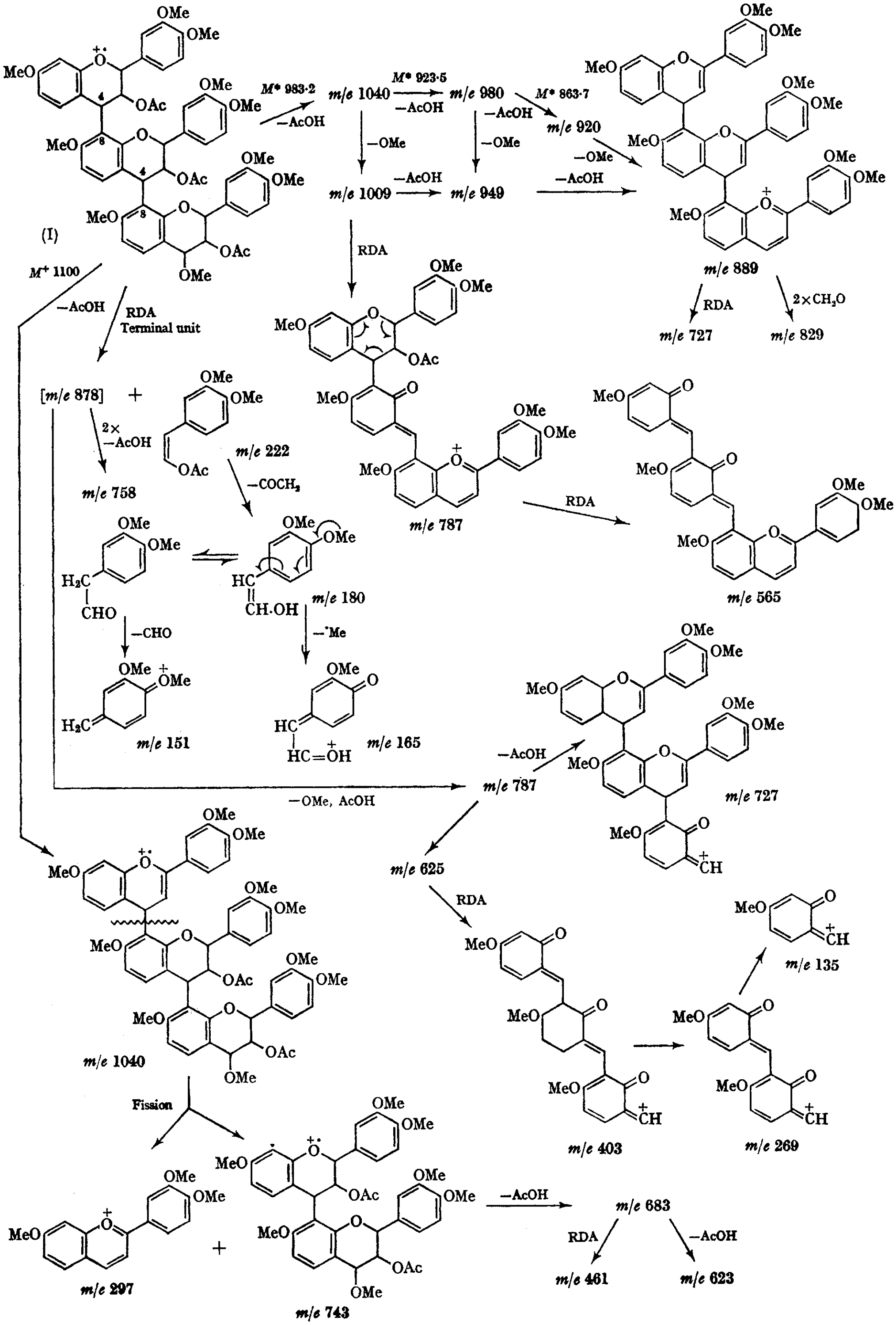
THE molecular gradation of oligomeric flavanoid components present in the heartwood of black wattle (*Acacia mearnsii*) contains an optically active condensed tannin.[1] This differs from the associated (+)-3′,4′,7-trihydroxyflavan-3,4-diol in that, on treatment with mineral acid, the yield of anthocyanidin chloride approximates to one third of that resulting from the monomer.[2] The molecular weights of the methyl ether and acetate, determined by ebulliometry, approach those of a trimer and thus supplement the above chemical evidence but, at best, the method gives only approximate values.

Introduction of mass spectrometry has overcome the difficulty of such approximate molecular weights, and was applied successfully in elucidating the structures of three bileucofisetinidins[3] and three biflavanols[4] from wattle heartwood and wattle bark, respectively. Extension of the method to the trimeric field has now resulted in an accurate molecular weight determination of a single component from the above trimeric tannin mixture. The molecular ion of the decamethyl ether triacetate derivative (M^+ 1100) lies well above the range of polymeric flavonoid compounds (524—804) previously studied by mass spectrometry. Peaks even in the upper range are well defined and of reasonable intensity and the spectrum (Figure) is rationalized readily.

The trimeric tannin mixture was re-isolated using chromatographic techniques similar to those already described.[3] Examination of the free phenolic form by paper ionophoresis[5] showed the presence of at least four bands. Methylation with diazomethane followed by repeated separation by preparative thin layer chromatography gave four fractions. One of these, [R_F 0·42 in methyl ethyl ketone: toluene (1 : 1 v/v)], migrated as a discrete spot in three different solvent systems. The molecular weight of this fraction (3 mg.) was M^+ 974. Other prominent peaks were at *m/e* 956 ($M^+ - 18$), 925 [$M^+ - (31 + 18)$], 795, 763, 583, 297, 180 and 151, the latter six representing stages of fragmentation due mainly to retro-Diels–Alder (RDA) fissions of the heterocyclic rings.[6]

Acetylation of the methyl ether gave a white solid (2 mg.), m.p. 113—115°. The mass spectrum (Figure) of this decamethyl ether triacetate (I) or (II), is rationalized in the Scheme and is based mainly on three retro-Diels–Alder processes, horizontal fragmentations, and loss of substituents. The increase in molecular weight on acetylation (from 974 to 1100) correlates with the introduction of three acetyl groups. The presence of a methoxyl at the C-4 position is inferred from fragments at 743, 683, and 623 as well as from other evidence below. Noteworthy is that the main peaks in the high mass range, *i.e.*, at *m/e* 1009, 949, and 683 contain the stable oxonium ion in the heterocyclic ring. Peaks at *m/e* 297, 180, and 151 (base peak) are also very prominent in the mass spectra of the corresponding derivatives of 3′,4′,7-trihydroxyflavan-3,4-diol,[7] and bileucofisetinidins.[3] Accurate mass measurement on the intense peak at *m/e* 683


(I)
M^+ 1100
M^* 983·2
−AcOH
m/e 1040
M^* 923·5
−AcOH
m/e 980
M^* 863·7
−AcOH
m/e 920
−OMe
−OMe
−OMe
m/e 1009
−AcOH
m/e 949
−AcOH
m/e 889
RDA
$2 \times CH_2O$
m/e 727
m/e 829
RDA
m/e 787
RDA
m/e 565
−AcOH
RDA
Terminal unit
[m/e 878]
+
m/e 222
2× −AcOH
m/e 758
$-COCH_2$
m/e 180
−CHO
−·Me
m/e 151
m/e 165
−OMe, AcOH
m/e 787
−AcOH
m/e 727
m/e 625
RDA
m/e 403
m/e 269
m/e 135
m/e 1040
Fission
m/e 297
+
m/e 743
−AcOH
m/e 683
RDA
−AcOH
m/e 461
m/e 623

gave 683·2506 in agreement with the calculated value of 683·2492 for $C_{39}H_{39}O_{11}$. The prominent peaks at *m/e* 183 and 185, recorded at a source temperature of 350°, cannot be rationalized on the above scheme. On spectra recorded at lower temperatures (220° and 260°) these had considerably lower intensities (10 and 13, and 41 and 38 respectively, as opposed to 82 and 79). Over this range the intensity of another intense peak, that at *m/e* 683, remained unchanged. These two peaks (*m/e* 183, 185) could arise from pyrolysis, particularly since they are absent from the spectrum of the decamethyl ether triol, M^+ 974.

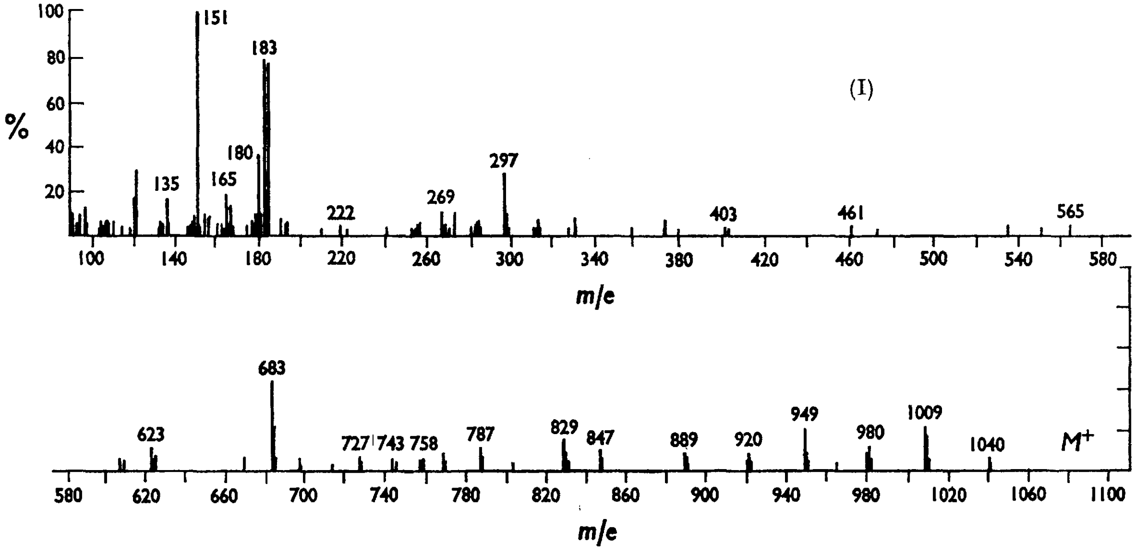
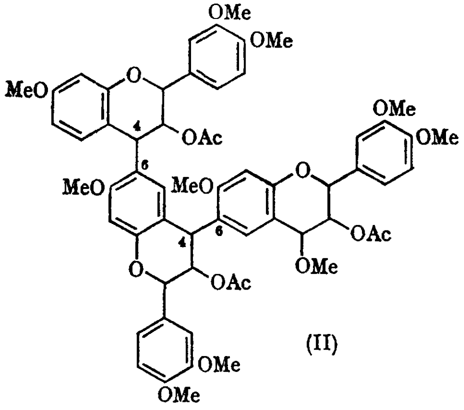


FIGURE. *Mass spectrum of decamethyl ether triacetate derivative of trileucofisetinidin.*

Mass spectrometry yields no decisive information regarding the two most likely alternative modes of link between the three C_{15} units. The 4,8-link in (I) has been drawn for the sake of convenience only. Evidence from the bileucofisetinidins[3] suggests that a 4,6-link as in (II) is the more likely. Other formulae containing 4,8- and 4,6-links within the same molecule are possible on the evidence presented.

The presence of a methoxyl group at C-4 of the terminal molecule is unusual although the ability of this benzylic hydroxyl to form ethers is well known[8] and can be demonstrated by paper chromatography of the free phenolic form of flavan-3,4-diols. Recent mass spectrometric investigations[7] also show that "pure" methyl ether derivatives of monomeric, as well as bimolecular, leucofisetinidins all have an additional $+14$ peak above the molecular ion due to C-4 methylethers.



(II)

Similar methylation of the tannin probably occurs during methanolic extraction of the raw material (free phenolic form).

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