CHAPTER IV

DISCUSSION

1. Structure of the New Naturally Occurring Eudesmane Derivative

The new naturally occurring eudesmane derivative was characterised as 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone. Its structure was elucidated from the ultraviolet, infrared, nuclear magnetic resonance and mass spectra, and its hydrolytic product.

The arguments for the elucidation of the structure of PI-1 are as follows :

Mass measurement of the molecular ion of the isolate established a molecular formula $C_{24}H_{36}O_8$, and of particular interest in the mass spectrum were the ions at *m/e* 217 ($C_{15}H_{21}O$) and *m/e* 131 ($C_6H_{11}O_3$). Whereas the latter suggested a polyfunctional ester unit, the former indicated the nucleus to be a sesquiterpene. Based on prior phytochemical work with *Pluchea* species it was considered that the nucleus might be of the cuauhtemone type. From the ir spectrum hydroxy, saturated ester and α,β-unsaturated ketone functionalities were evident. From the λ_{max} at 258 nm it was apparent that the ketone was α,β,β-trisubstituted (calc. 254 nm), and this was confirmed by the absence of any olefinic proton below 5.4 ppm.

The structure of the isolate was established by the complete assignment of the 400 MHz proton nmr spectra through use of double resonance and Inter Nuclear Double Resonance (INDOR) techniques. Preliminary examination of the spectrum in comparison with published nmr spectral data for compounds in this series, indicated the presence of two acetates at δ 2.09, quaternary methyl groups at C-10 (0.98 ppm) and C-4 (1.28 ppm) and two olefinic methyl groups at δ 1.86 (13-H_3) and δ 2.10 (12-H_3). Two other three-proton singlets in the spectrum (δ 1.26 and 1.66) were assigned to groups in an ester side chain. These data suggest a cuauhtemone derivative esterified with a side chain having the molecular formular $C_9H_{13}O_5$. The presence of a downfield quartet at δ 5.24 coupled to the three-proton doublet ($\underline{J}$= 6 Hz) at δ 1.26 was confirmed through irradiation, and indicated that this would be a 2,3-diacetoxy-2-methyl butyryl unit. It therefore remained to establish the location of the acylating unit.

Mild hydrolysis of the location and stereochemistry of the hydroxy groups. This stereochemical array was confirmed through irradiation in the spectrum of 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone, of the apparent triplet ($\underline{J}$= 3 Hz) at δ 5.02 which simplified the complex multiplet in the region δ 1.76-1.86. Irradiation at δ 1.81 confirmed the coupling to an equatorial 3β-H, and also permitted assignment of the 1α-H to a slightly broadened doublet ($\underline{J}$= 15 Hz) at δ 1.49 and the 1β-H to a sharp doublet at δ 1.33.

Two aliphatic doublets (J= 15 Hz) at δ 2.17 and 2.25 were assigned to the 9-H_2. Irradiation at δ 2.17 collapsed the signal at δ 2.25 to a singlet and this coupling was further substantiated through INDOR experiments monitoring each proton successively. Individual assignment of the 9α-H and 9β-H resonances was based initially on prior data for compounds in this series.

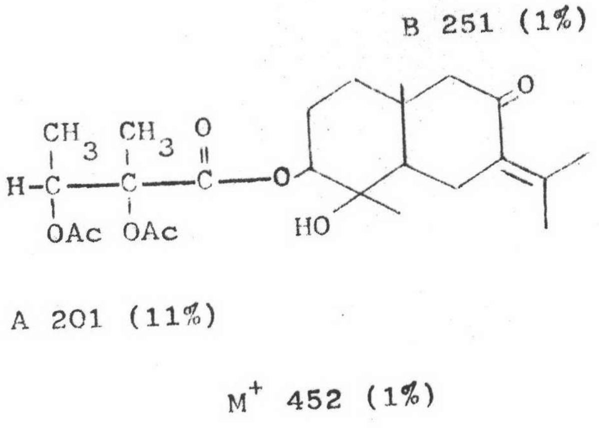
The slightly broadened doublet of doublets (J= 4, 13 Hz) at δ 1.92 was assigned to the 5α-H and irradiation collapsed the signal at δ 3.01 to a doublet (J= 15 Hz), which must therefore be the 6α-H. Although significant changes were also observed in the region δ 2.15-2.27, it was only through irradiation at δ 2.17 that the signals at δ 3.01 and 1.92 were collapsed to doublets (J= 4 Hz) permitting the assignment of the 6β-H. One of the 2-H_2, both of which must be in the region δ 1.76-1.86 remained to be assigned. Since irradiation at δ 1.49 (1α-H) collapsed the region around δ 1.84 more than irradiation at δ 1.33 the 2β-H must be in this region and the 2α-H at about δ 1.81. Because of the non-first order nature of this part of the spectrum these values must be considered approximate.

That the ester group is at C-3 and the hydroxy group at C-4α (rather than in the side chain), is apparent from the 3β-H at δ 5.02 and the 4-CH_3 at δ 1.28. These data are in agreement with those for a 4-hydroxy derivative and not for the corresponding 4-acetyl derivative where these signals are observed at δ 5.86 and 1.60, respectively. The isolate therefore has the structure 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone.

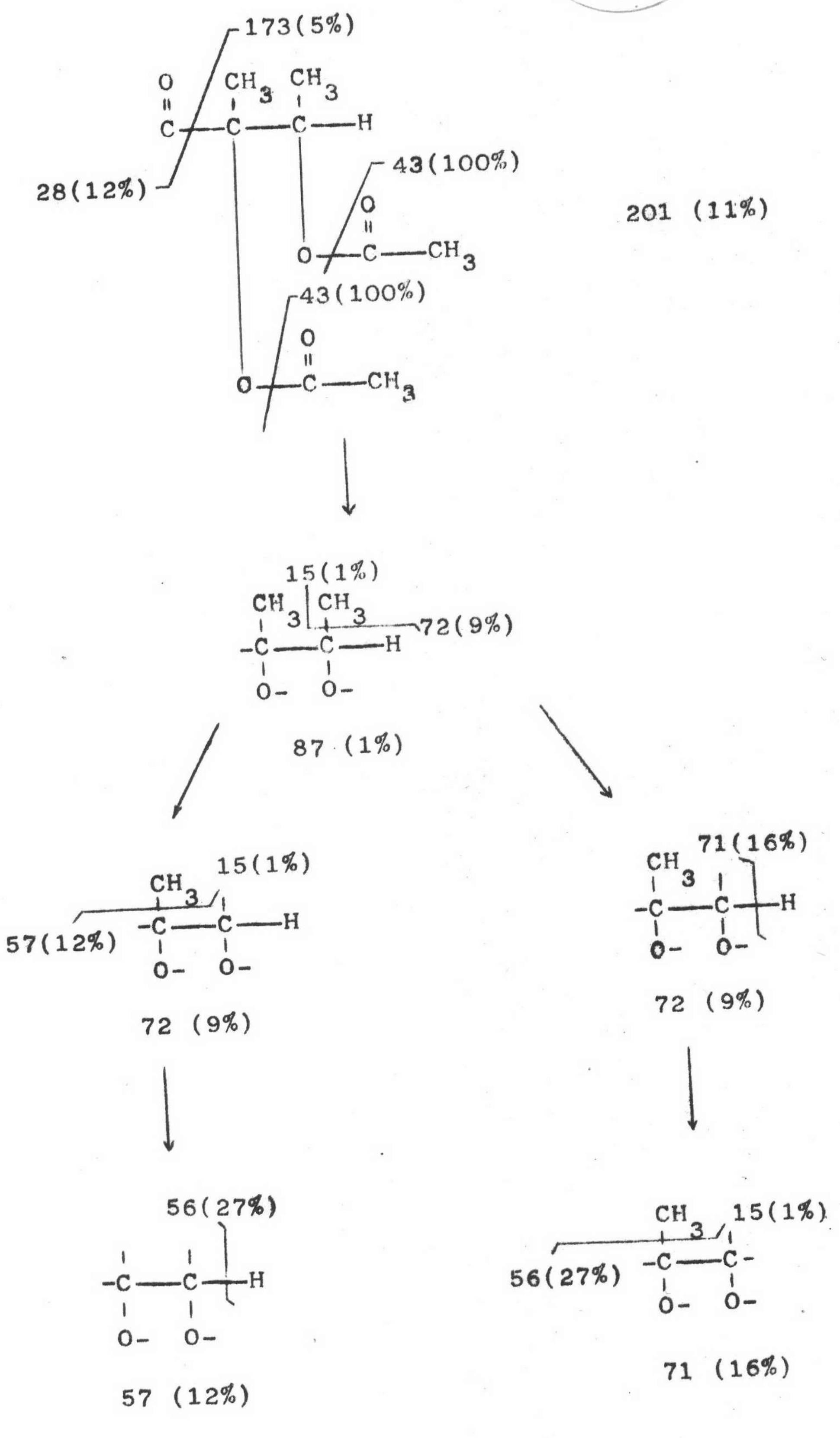
One further interesting observation was made, namely that the 1α-, 5α- and 9α- resonances were all slightly broadened. This was particularly clear for the 1- and 9- methylene protons where direct comparison could be made with the corresponding β- protons which were invariably sharp.

It was suggested that this broadening to be due to weak coupling with the 10-methyl group. Indeed the resolution enhanced 400 MHz spectrum clearly shows this methyl group to be broadened significantly on comparison with the 5'-H_3.

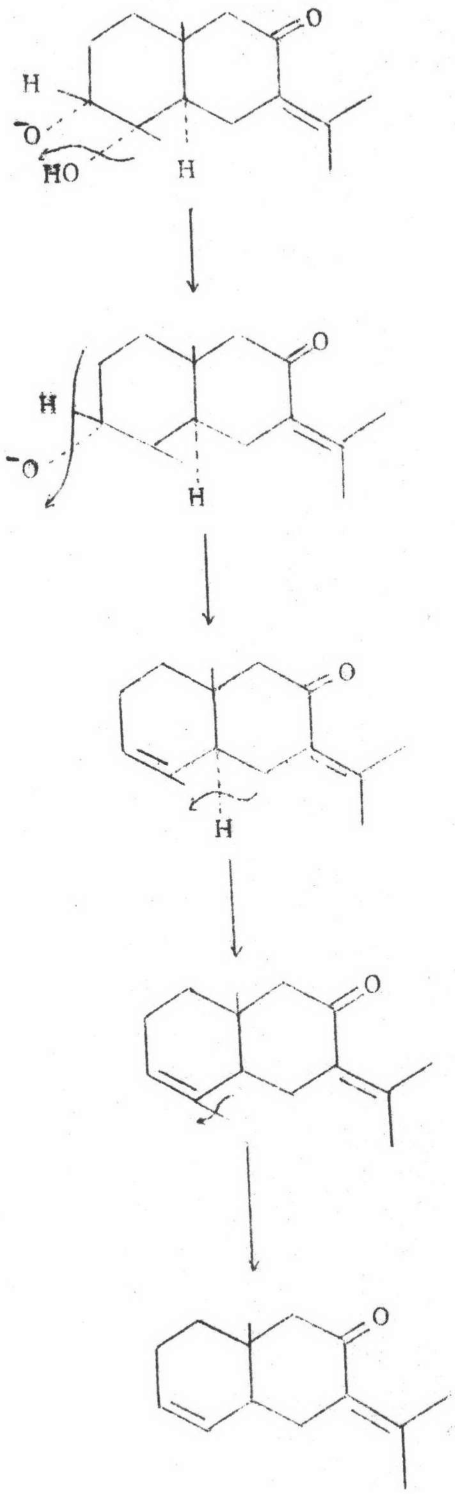
From mass spectrum, the fragmentation of 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone is proposed corresponding to spectrum peaks as follows :



Fragmentation of A



Fragmentation of B



2. The Chemotaxonomic Significance of Eudesmane in *Pluchea*

Chemical investigations have indicated that the Inuleae contains a considerable range of secondary constituents, some of which are undoubtedly unique to the tribe. Sesquiterpenes of eudesmane type are widespread in plant. They are found in Gymnosperms and Angiosperms both in Monocotyledonae and Dicotyledonae. Some derivatives occur in lower plants, liverworts and marine algae. Only one such sesquiterpene (4,11-epoxy-*cis*-eudesmane) was also reported in animal kingdom.

Eudesmane compounds were reported in various species of Compositae. In tribe Inuleae they were found in *Jasonia glutinosa* DC., *Pluchea chingoyo* DC., *P. dioscorides* DC., *P. foetida* (L.) DC., *P. odorata* Cass., *P. rosea* Godfrey, *P. suaveolens* Kuntze. In this present investigation *P. indica* Less. is shown to have 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone. This result indicates that most probably other species of this genus contain eudesmane derivatives.

The presence of cuauhtemone in all 7 species of *Pluchea* reported opens an important area for future investigation.