



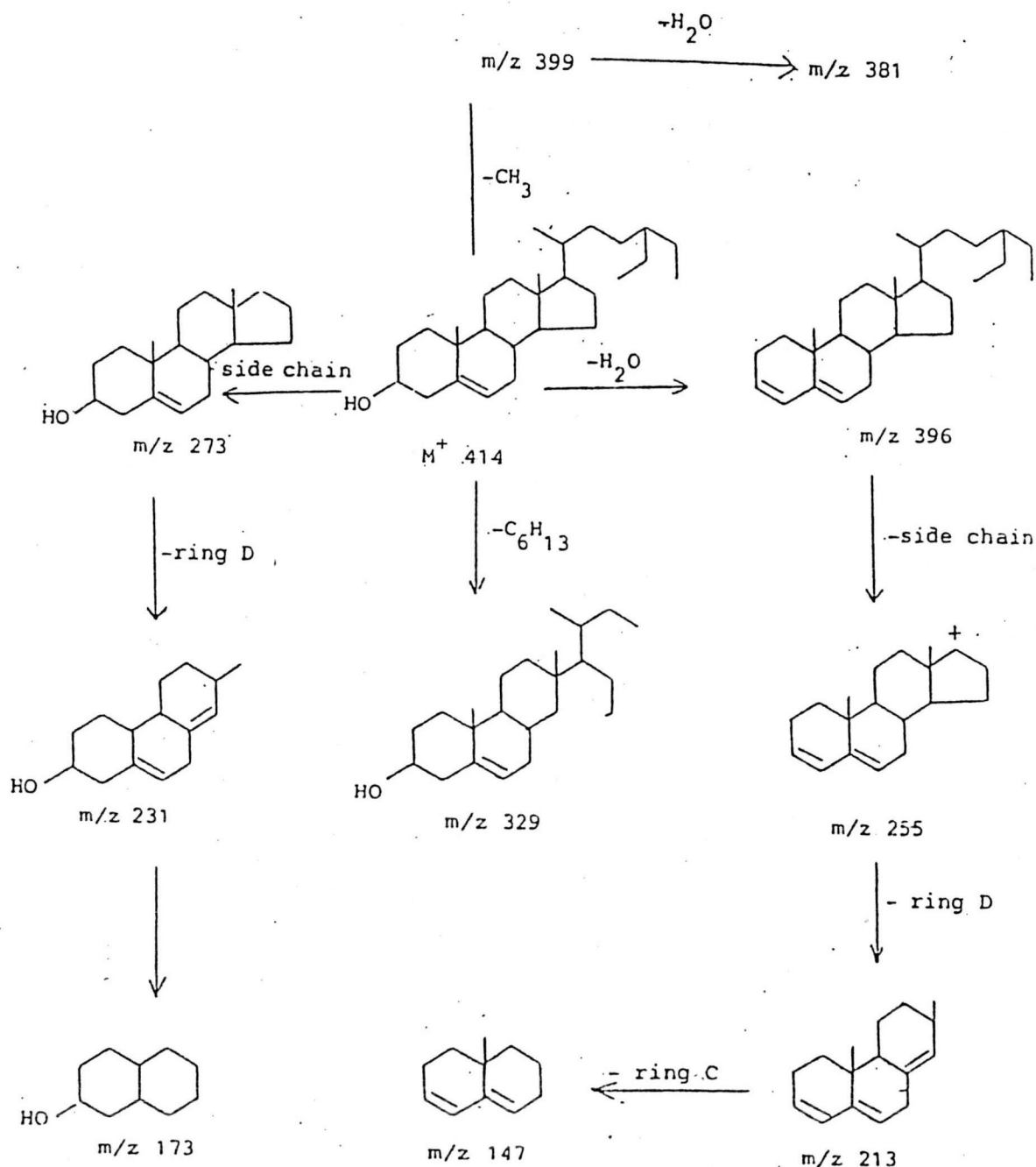
CHAPTER IV

DISCUSSION

The residue from the petroleum ether extract of dried whole plant of Eupatorium adenophorum Spreng. was separated into three pure components by column chromatography as described in the experimental chapter.

The first component "EA-1" was obtained as a white powder, its IR spectrum (Fig. 3.6) revealed the presence of hydroxy and olefinic functional groups. The electron impact mass spectrum (EIMS, Fig. 3.8) exhibited a weak parent ion at m/z 414, corresponding to molecular formula $C_{29}H_{50}O$ and the intense $M-H_2O$ (m/z 396) peak indicated a characteristic of $\Delta^5-3-\beta$ -sterol type of compound (142). The proposed fragmentation pattern (143,144) was shown on page 94. The 1H -NMR spectrum (Fig. 3.7) was identical to those obtained from a sample of β -sitosterol, which had been isolated from Typha elephantina, (141) and also were in accord with previous published data for this sterol (145). Thus, the first component "EA-1" is β -sitosterol.

The second component "EA-2" was obtained as a white feather-like crystal. Mass spectrometric analysis of this compound indicated a molecular ion at m/z 234 (Fig. 3.20), corresponding to an elemental composition of $C_{15}H_{22}O_2$. Intense absorptions in the UV (MeOH) spectrum at λ_{max} 242



nm ($\log \epsilon$ 3.86, Fig. 3.9) and in the IR (KBr) spectrum at ν_{\max} 1725, 1710, 1650 cm^{-1} (Fig. 3.11) suggested the presence of both saturated and α , β -unsaturated ketonic groups. The ^1H NMR spectrum of EA-2, obtained in CDCl_3 (Table 5., Fig. 3.12), indicated the presence of an olefinic hydrogen (δ 6.35, d, $J = 1.5$ Hz), both allylic (δ 1.73, d, $J = 1.5$ Hz) and aliphatic (δ 1.02, d, $J = 6.4$ Hz) methyl group, and an isopropyl group (δ 0.88, d, $J = 6.1$ Hz; 1.08, d, $J = 6.1$ Hz; 2.00, m). Two geminally coupled methylene groups (δ 2.08, 2.20, $J_{\text{gem}} = 8.8$ Hz; and δ 2.54, 2.81, $J_{\text{gem}} = 16.6$ Hz) were also observed in the molecule.

The ^{13}C -NMR spectrum of EA-2 (Table 5.) substantiated these structural implications and, in addition, showed resonances for a saturated cycloheptenone (δ 212.87), an α -substituted cycloheptenone (δ 198.10) and six methine carbon signals including a vinylic CH (δ 146.89) and an isopropyl methine (δ 31.78). From the structural elements presented and the coupling pattern of the homonuclear COSY spectrum (Fig. 3.14-3.16) of an unsaturated azulene skeleton were suggested for EA-2 structure. The ^1H - ^1H COSY spectrum (Fig. 3.14-3.16) indicated a long-range coupling between the allylic methyl protons (δ 1.73) and the vinyl hydrogen 1-H (δ 6.35), and the coupling of the latter with the anellated methine, 2-H (δ 3.22). From the relatively small (>4 Hz) coupling between 2-H and 6-H (δ 2.28) a cis junction between the five and seven-membered rings was indicated. An additional

small coupling (>1 Hz), observed only in the COSY spectrum for the 2-H signal with 3-H at δ 2.05, indicated the near 90 torsion angle between these two hydrogens. Consequently, the orientation of the methyl group at C-3 was deduced to be the same as that of the hydrogens at anellation positions (2-H and 6-H). The 8-H₂ methylene protons showed a characteristic coupling pattern; the signal at δ 2.03 was a doublet of doublets, while the geminally coupled signal at 2.20 was a doublet, since only one of these two signals shows coupling with 7-H (δ 2.05). The dd pair of 5-H₂ at 2.54 and 2.81, however, shows strong geminal coupling, and both signals are coupled with the resonance for 6-H at 2.28.

The relative stereochemistry of the substituents as well as certain conformational features of EA-2 were further established by NOE experiments. Irradiation of 2-H at δ 3.22 enhanced the C-3 methyl and 6-H methine signals, thereby supporting the cis orientation of these three substituents. Irradiation of 1-H at δ 6.35 resulted in area increases at δ 2.05 (3-H) and δ 1.73 (10-CH₃). No NOE effect was observed between 1-H and 3-CH₃. Separate irradiations of the two dd signals of 5-H₂ established the stereotopical nature of the two non-equivalent hydrogens. Thus, irradiation of the signal at δ 2.81 resulted in an area increase for the isopropyl methyl groups, but irradiation of the signal at δ 2.54 did not result in any NOE effect being observed.

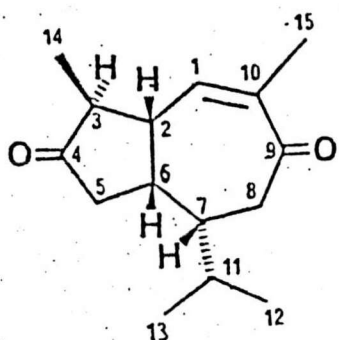
Further evidence for the unusual carbon framework and substitution pattern of EA-2 came from selective INEPT (Fig. 3.19) experiments which also permitted the unambiguous assignment of the ^{13}C NMR spectrum. The APT spectrum of EA-2 showed four methyl (δ 15.45, 19.97, 20.28 and 20.93), two methylene (δ 41.04, 42.18), six methine (δ 28.10, 31.78, 39.24, 42.18, 63.90 and 146.89) and three quaternary carbons (δ 136.00, 198.10, and 212.87), of which the latter two could be assigned as carbonyl carbons. Irradiation of 10- CH_3 resulted in enhancements of δ 198.10 and 146.89 which could be assigned as C-9 and C-1, respectively, and irradiation of the isopropyl methyl group enhanced the aliphatic methine (C-7) at δ 28.10. Magnetization transfer via irradiation of 2-H confirmed the position of the carbonyl function in the five-membered ring leading to enhancement at δ 212.87 (C-4), δ 136.00 (C-10), δ 41.04 (C-5) and δ 28.10 (C-7). Finally, irradiation of 1-H enhanced C-9 at δ 198.10, thereby, confirming the carbonyl placement in the seven-membered ring, C-3 at 63.90, C-6 at δ 39.24 and C-15 at δ 15.45 (Fig. 3.19, p. 146). The assignments for the protonated carbons obtained from the selective INEPT experiments were in agreement with the HETCOR spectrum (Fig. 3.17-3.18) of EA-2, and the complete assignments are shown in Table 5.

Unlike saturated ketone or isolated olefins, α , β -unsaturated ketones are regarded as a class of inherently dissymmetric chromophores, consequently no sector rules were

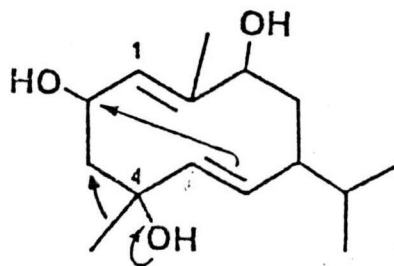
applicable for the determination of the absolute configuration of the chiral center of EA-2. The sign of the $n-\pi^*$ (R-band, 320-350 nm) and $\pi-\pi^*$ (K-band, 220-260 nm) transitions of trans-enones, however, have been correlated with the sense of helicity for this dissymmetric type chromophore. Thus, the R-band is positive and the K-band is negative in the case where the helicity of the chromophore is skewed in a left-handed helix; and they are opposite when the chromophore helicity is right-handed. Since EA-2 exhibited a CD spectrum characteristic for the presence of a right-handed helicity of the trans-enone chromophore, the absolute configuration of the attached C-2 chirality center was established at S. Experimentally, the CD values in methanol were $[\theta]$ +2150 and -840 at 248 and 300 nm, respectively. On this basis, and according to the prior determination of the relative steric positions of 2-H, 6-H, 3-CH₃ and 7-CH₃, the absolute configurations of the stereocenters of EA-2 are proposed as 2S, 3S, 6S, and 7R.

Biogenetically, EA-2, named "EUPATORENONE", represents a new class of sesquiterpenoids in which the C-4 methyl group has apparently migrated to C-3. One may envisage this to occur from a cis,-trans-germacradiene such as A at the time of cyclisation.

Eupatorenone was evaluated for cytotoxic in the KB and P-388 test systems according to established protocols, but was inactive.



Eupatorenone



A

The third component "EA 3" was obtained as semisolid substance with characteristic odour. The $^1\text{H-NMR}$ spectrum of EA-3 revealed the presence of many CH_3 and CH_2 groups of triterpene (Fig. 3.21). Since its structure is not inspired, the further structure elucidation is not performed.