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INSECT ANTIFEEDANTS FROM TROPICAL PLANTS I. STRUCTURE OF DUMSIN

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Introduction

There has been a lot of interest in the effect plant metabolites have upon man, and most cultures have long had lists of plants with medicinal qualities.[1,2] Many of the secondary metabolites found in plants are believed to have originally occurred as a result of the pressures of natural selection during the coevolution of insects and plants.[3] It is possible that some of the medicinal compounds found in plants were originally formed in response to evolutionary pressures from insects, and that their ability to effect man is rooted in the similarity of primary metabolism among animals. Hence, the well documented sources of medicinals from plants may also serve as sources of insecticidal compounds. For this reason the insect active properties of the rootbark extract of the East African medicinal plant "Msinduzi" (Swahili), tentatively identified as Croton jatrophoides Pax.[4], were examined. The native people of East Africa use the roots as a remedy for colds and for stomach ache.[5]

Results and Discussion

The bitter rootbark was first extracted with methanol and found to be active in an artificial diet feeding assay using lepidopteran larvae.[6] After solvent removal the extract was sequentially partitioned between water and: hexanes, chloroform and ethyl acetate. Subsequent bioassays identified the chloroform portion of the extract as containing the active constituents. Repeated chromatography of this portion of the extract on normal and reverse phase silica gel led to the isolation of eight new compounds active in our bioassay. The isolation and structural elucidation of one of these antifeedant limonoids will be reported.

Compound I was isolated as a white crystalline solid (mp 193°C; $[\alpha]^{20}$ +18 (C = 0.08, $CHCl_3$)). In Beam EI-MS indicated a parent ion of 642 amu. The molecular formula suggested by this mass, $C_{35}H_{46}O_{11}$, was consistent with all ^1H

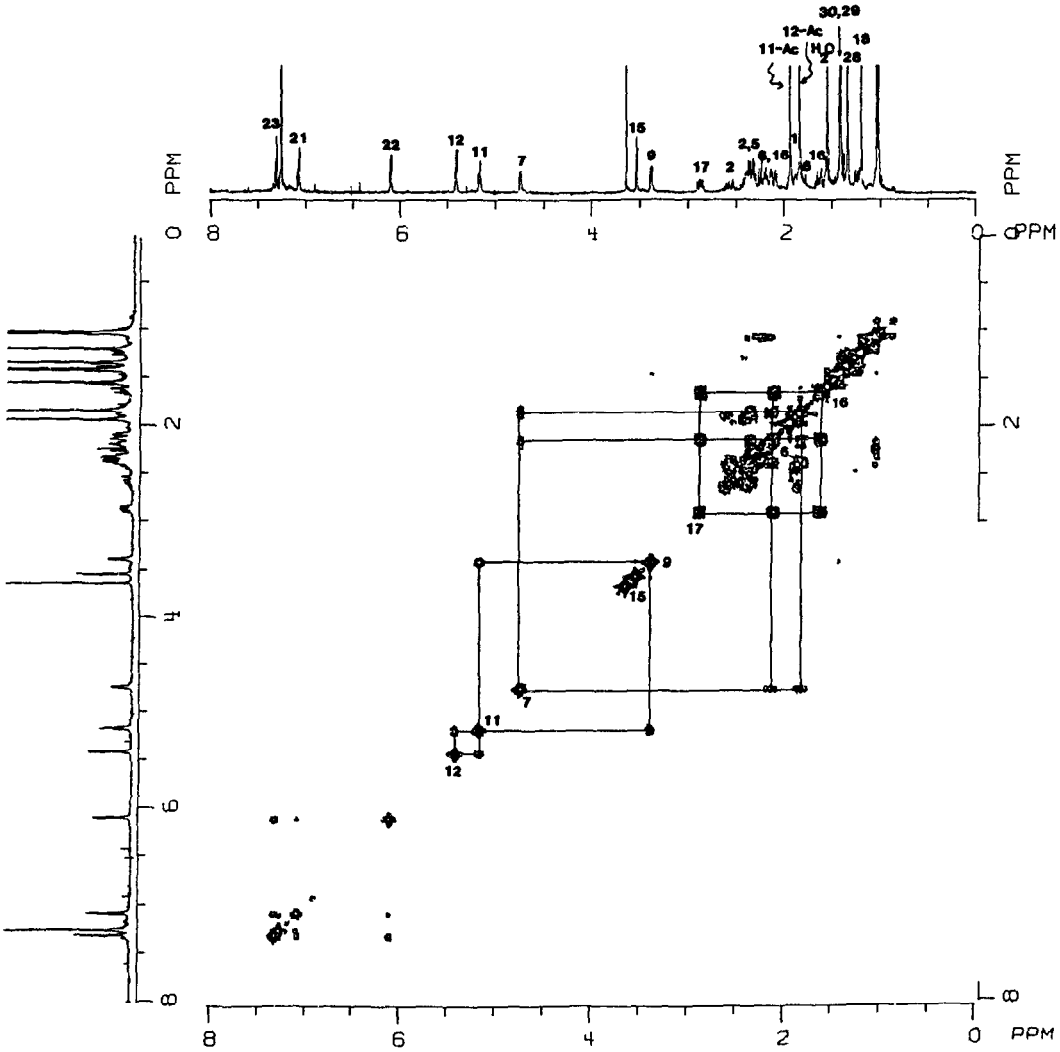
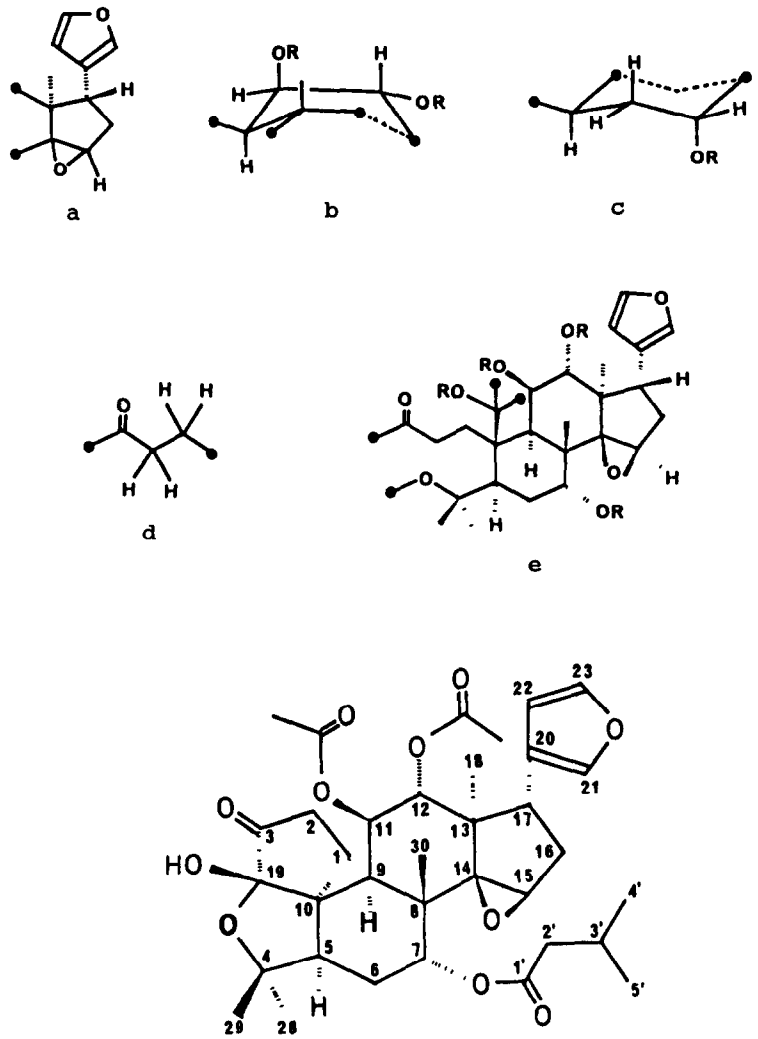


Figure 1. The 2D ^{1}H COSY NMR spectrum of I at 300 MHz ($CDCl_3$).

NMR spectral information. The ^{1}H-^{1}H 2D-COSY NMR spectrum enabled the identification of an isopentanoate group through couplings obscured in the ^{1}H 1D-NMR spectrum; 2 coincident methyl resonances (1.04 ppm, d; 6.8 Hz, 6H) coupled to a methine ^{1}H at 2.2 ppm (m). The complex multiplet at 2.2 ppm also contained the two methylene ^{1}H resonances alpha to the isopentanoate's carbonyl group. Two acetate groups were also observed (1.94 ppm, s, 3 H; 1.84 ppm, s, 3 H; IR 1730 cm^{-1}, br). The presence of a beta-substituted furan (7.32, 7.07, 6.01 ppm; UV (CH_3OH) 214 nm, ϵ = 3900) was confirmed through the couplings of



Dumsin

resonances observed in the 2D ^{1}H-^{1}H COSY NMR spectrum and through the comparison of chemical shifts to those of model compounds such as surenin.[7] The presence of the β-substituted furan suggested compound I was a tetranortriterpene of the limonoid type. Also, isopentanoate groups, which are uncommon in most classes of natural products, have been found in several other limonoids such as salannoland rohitukin.[8,9] The proton at 2.87 ppm was observed in the 1D spectrum to be coupled to the two protons of an adjacent methylene (2.11 ppm, dd; 14, 6 Hz: 1.61 ppm, dd; 14, 11 Hz). It was evident that these two protons were on a

single methylene, rather than on two separate carbons, from their large 14 Hz geminal coupling. The long-range W coupling of the axial methyl at 1.20 ppm (s, 3H) to the adjacent proton at 2.87 ppm (dd; 6, 11 Hz) observed in the 2D ^{1}H-^{1}H COSY NMR spectrum suggested this ^{1}H was trans axial to the methyl. Coupling of the methine proton at 3.54 ppm (br s) to the shifts at 2.11 ppm and 1.61 ppm could not be clearly observed in the 1D spectrum yet were visible in the 2D-COSY NMR spectrum (see Figure 1). While it was not possible to measure the precise values of these coupling constants, there are several reports of vicinal couplings of their approximate magnitude in the D-ring of limonoids.[10,11,12] An examination of the literature identified the protons of this portion of the compound as characteristic of the D-ring of the trichilins (see partial structure a).[13,14] Another fragment constructed consisted of a proton at 3.38 ppm (d; 4.8 Hz) observed in the 2D ^{1}H-^{1}H COSY NMR spectrum to be long range coupled to an axial methyl at 1.42 ppm. This is consistent with the methyl and the proton being trans to one another so as to be in a W configuration. The proton at 3.38 ppm was also coupled (4.5 Hz) to a proton as 5.17 ppm (dd; 4.5, 4.0 Hz). The magnitude of this coupling, and the proton at 3.38 ppm being axial, suggests the proton at 5.17 ppm to be equatorial. The proton at 5.17 ppm was further coupled to a signal at 5.41 ppm (d; 4.0 Hz, see Figure 1). These data are consistent with the partial structure b. The proton at 2.37 ppm (dd; 11, 6 Hz) was placed vicinal to two geminal methylene protons (2.12 ppm, ddd; 14, 11, 4 Hz: 1.81 ppm, ddd; 14, 6, 2 Hz) which in turn were coupled to a methine proton at 4.74 ppm (dd, 4, 2 Hz). These observations are consistent with a six membered ring in a chair conformation as shown in partial structure c. The couplings of the proton at 2.57 ppm (ddd; 11, 9, 3 Hz) identified the three other proton resonances associated in partial structure d. The structural features described thus far were assembled to give partial structure e using the trichilins as a model. However, it was not possible to assign the relative positions of the two acetate groups and the isopentanoate group due to the close spectral similarities of the possible isomers. The question of the stereochemistry of the modified limonoid A ring was also very difficult to obtain because of the number of quaternary centers involved in this portion of the compound. It was decided these remaining questions could be best answered by undertaking an x-ray diffraction study of I.

Compound I crystallized in the orthorombic crystal class. Accurate lattice parameters, determined by a least squares fit of fifteen diffractometer measured 2θ-values, were a = 11.1066(20), b = 13.1509(33), and c =23.8662(45) Å. The systematic extinctions (0k0, k=2n+1; 001, l=2n+1), crystal density, and optical activity were uniquely accommodated by space group $P22_12_1$ with an asymmetric unit of $C_{35}H_{46}O_{11}\cdot H_2O$. All unique diffraction maxima with $2\theta \leq 140°$ were

collected on a computer controlled four circle diffractometer using graphite monochromated Cu Kᾱ radiation (1.54178 Å) and variable speed 1° ω-scans. Of the 2678 reflections measured in this fashion, 2416 (90%) were judged observed ($F_o \geq 3\sigma(F_o)$) and used in subsequent calculations[15] A phasing model was found using a multisolution direct methods approach and tangent formula recycling of a plausible molecular fragment.[16] Hydrogens were located in a difference electron density synthesis. The final R-factor is 0.056 for a model with anisotropic nonhydrogen atoms and isotropic hydrogen atoms.[17] Figure 2 is a computer generated perspective drawing of the final x-ray model of I. Hydrogens have been omitted for clarity, and, since the x-ray experiment did not define the absolute configuration, the enantiomer shown is an arbitrary choice.

Carbonyl groups in five-membered rings show a Cotton effect of the same sign as carbonyl groups in six-membered rings of similar configuration and generally have about 1/5 of the magnitude of the Cotton effects observed with carbonyls in six-membered rings.[18] A negative Cotton effect could be seen at a wavelength (CD (CH_3OH) 308 nm, ϵ = 0.97 degree M^{-1} cm^{-1}) typical for the n→π* transition of a simple cyclopentyl ketone. The octant rule suggests the absolute configuration of I is as shown in Figure 2. There are potential problems in predicting the Cotton effects of five-membered ring ketones. However, these

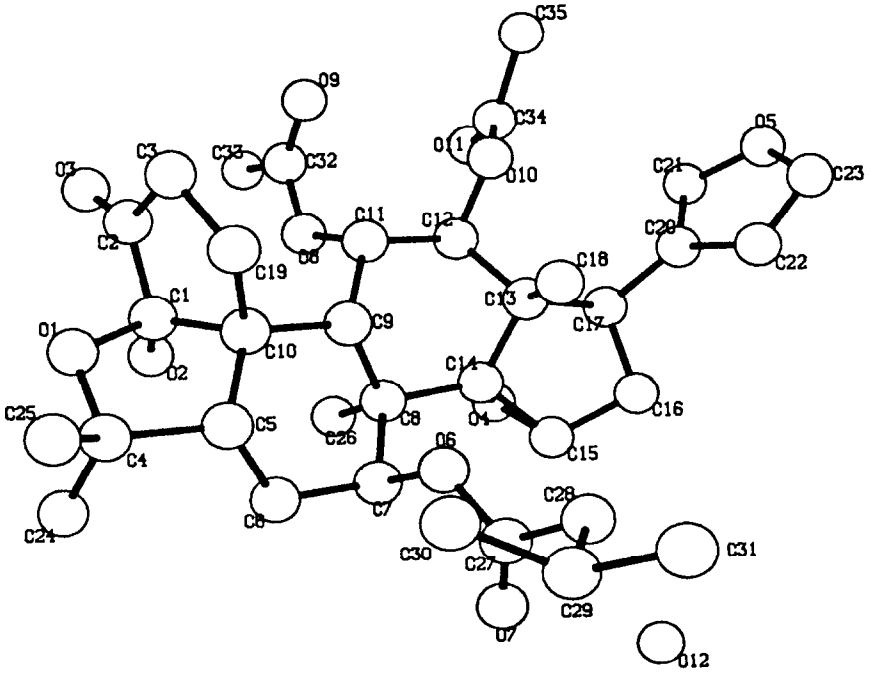


Figure 2. The computer generated perspective drawing of the x-ray model of I.

problems usually involve rings that are held twisted out of plane by a rigid ring system.[18] The absolute stereochemistry of I as predicted here and shown in Figure 2 is consistent with other limonoids.

Conclusion

Compound I represents a new structure and has been named Dumsin based on the Swahili name for the medicinal root bark.[5] This compound joins other tetranortriterpenoids in a class of compounds currently attracting attention due to their antifeedant activity on insects.[19] The cyclopentyl ether portion of compound I differs from the common limonoid limonin in that the ether bonds appear to involve a C4-C19 ether linkage rather than a C4-C1 ether linkage. While this type of ether linkage is uncommon it can still be found in limonoids such as jangomolide[20] and cycloepiatalantin.[21] As this is the first report of limonoids from the family Euphorbiaceae their isolation has created doubts as to the identification of the plant material. Limonoids are usually found in the Rutaceae and Meliaceae families of plants.[22] However, there have been recent reports of limonoids from the Cneoraceae and Flacourtiaceae families.[20] It could very well be limonoids are more widespread than has been previously realized. We are now attempting to recollect C. jatrophoides to permit the isolation of additional metabolites and to secure the identification of the plant material.

Experimental

All NMR spectra were run on a Nicolet spectrometer equipped with an Oxford superconducting magnet operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C and were in $CDCl_3$ unless otherwise specified. All shifts were reported using $CDCl_3$ as an internal reference (7.24 ppm, 77.0 ppm). All pulse sequences used were those commercially available and were supplied by the spectrometer's manufacturer. Specific rotations were obtained with a Perkin-Elmer 241 MC polarimeter. Mass spectra were obtained using a Hitachi RMU 6-MG spectrometer in an electron impact mode (EI-MS). IR spectra were obtained on a Perkin-Elmer model 1310 spectrometer in $CHCl_3$. Circular dichroism (CD) measurements were obtained on a JASCO J-40 spectropolarimeter (c = g/100 ml). UV spectra were acquired on a Hitachi 100-80 Spectrometer in ethanol. The x-ray data were collected on a Symtex Pz_1 diffractometer, Department of Chemistry, Cornell University. Experimental Data have been deposited.[23]

Plant material: A collection of root bark of the East African plant Msinduzi (Swahili) near Mombasa, Kenya, tentatively identified as Croton jatrophoides, was extracted with methanol at room temperature. The methanol was removed and

evaporated and the resulting oil was partitioned between water and: hexanes, chloroform and ethyl acetate. A bioassay using lepidopteran larvae of the pink bollworm Pectinophora gossypiella, raised on an artificial diet incorporating fractions of the extract, identified the chloroform portion of the extract as retaining the biological activity.[4] This fraction was further divided into 6 fractions using normal phase silica gel open column chromatography. Subsequent bioassays showed fraction 5 to be active. This fraction was then subjected to low pressure liquid chromatography with reverse phase silica gel ODS-18 to form 9 fractions. Fraction 9 contained pure compound I and showed very strong activity in the artificial diet assay.

Dumsin (I): Isolated as a white crystalline solid, mp 193°C; ^{1}H NMR ($CDCl_3$, ppm) 7.32 (m), 7.07 (m), 6.01 (m), 5.41 (d; 4 Hz), 5.17 (dd; 4.0, 4.5 Hz), 4.74 (dd;4, 2 Hz), 3.54 (br s), 3.38 (d; 4.5 Hz), 2.87 (dd; 11, 6 Hz), 2.57 (ddd; 11,9, 3 Hz), 2.38 (m), 2.37 (dd; 11, 6 Hz), 2.22 (m), 2.12 (ddd; 14, 11, 4 Hz), 2.11 (dd; 14, 6 Hz), 1.94 (s; 3H), 1.88 (m), 1.84 (s; 3H), 1.81 (dd; 14, 6.2 Hz), 1.61 (dd; 14, 11 Hz), 1.43 (s; 3H), 1.42 (s; 3H), 1.34 (s; 3H), 1.25 (m), 1.20 (s; 3H), 1.04 (d; 6 Hz, 6H); IB-MS m/z 642 M^+), $C_{35}H_{46}O_{11}$; IR ($CHCl_3$) 3505, 1754, 1730, 1240 cm^{-1}; UV (EtOH) 214 nm (ϵ = 3900); CD (CH_3OH) 308 nm (ϵ = 0.97 degree M^{-1} cm^{-1}).

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