5 m and 25 m × 0 2 mm immobilized OV-1 fused silica capillary columns, programmed from 100° to 300° at 4°/min The sample was loaded onto the column using an air-cooled, on-column syringe injector Carrier gas was He at a mean flow rate of 100 cm/sec, FID was at 300° Peak areas were determined by reporting integrator and are uncorr for relative response for esters over the C_{40}–C_{60} range GC/MS was carried out using a quadrupole filter instrument operating at 70 eV, 300 μA electron energy and an ion-source temp of 200° Other mass spectrometer parameters were chosen to maximize high mass sensitivity Spectra were taken at 2 8 sec intervals The capillary column was coupled to the mass spectrometer ion-source via an open-split interface heated maximally at 285° The approximate amount of each ester within a homologue was determined by integration of the ion chromatograms for the $[RCO_2H_2]^+$ fragments CI probe MS were obtained using CH_4 reactant gas at 0 6 Torr and an evaporation temp of 300°

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24β-ETHYLSTEROLS, *n*-ALKANES AND *n*-ALKANOLS OF *CLERODENDRUM SPLENDENS*

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Key Word Index—*Clerodendrum splendens*, Verbenaceae, 24β-ethylsterols, 24β-ethylcholesta-5,22*E*,25(27)-trien-3β-ol, 25(27)-dehydroponiferasterol, clerosterol, 25(27)-dehydrochionasterol, *n*-alkanes, *n*-alkanols, fatty alcohols, sterols

Abstract—The sterols of *Clerodendrum splendens*, an angiosperm belonging to the family Verbenaceae, were found to possess a 24β-ethyl group No other sterols were detected The major sterol was 24β-ethylcholesta-5,22*E*,25(27)-trien-3β-ol [also known as 25(27)-dehydroponiferasterol] A very small amount of what may have been its 22-dihydroderivative, clerosterol [also known as 25(27)-dehydrochionasterol] was also found The dominant *n*-alkane was C_{29} (*n*-nonacosane) and the dominant *n*-alkanol was C_{28} (*n*-octacosanol)

INTRODUCTION

In the great majority of mature angiosperms which have been investigated the dominant sterols possess a 24α-alkyl group [1] While smaller amounts of 24β-methylsterols often occur, 24β-ethylsterols have been found only rarely Examples include the Δ⁵-24β-ethylsterols of *Kalanchoe daigremontiana* [2], *Conopharyngia durissima* [3], *Enhydra fluctuans* [4, 5], *Cucurbita maxima* [6] and various species belonging to the genus *Clerodendrum* [7–13] Δ⁷-24β-Ethylsterols are well described in the family Cucurbitaceae [14–20] Interestingly, 24β-ethylsterols frequently appear to be unaccompanied by 24α-ethylsterols or by 24α- or 24β-methylsterols (in cases where the configuration at C-24 has been firmly established) However, there have been reports of the presence of sitosterol (without ^1H NMR substantiation of configuration) along with 24β-ethylsterols in the roots of *Clerodendrum paniculatum* and *Clerodendrum colebrookianum* [11] and the flowers of *Clerodendrum infortunatum* [12, 21] It has also been reported that the leaf fat (which was 4 1 % of the leaf material) of *Clerodendrum inerme* yielded two isomeric sterols with empirical formulae $C_{27}H_{46}O$, one of which was presumed to be cholesterol [22, 23]

Clerodendrum splendens, a native of Sierra Leone, became available to us through the kindness of Dr Donald G Huttleston This particular plant does not appear to have been previously investigated and it offered an

opportunity to examine further (a) whether 24β-ethylsterols are characteristic of the genus *Clerodendrum*, (b) if so, whether they would have a $\Delta^{25(27)}$-bond of biosynthetic significance [1, 2, 9, 24–26], and (c) whether other 24-alkyl or 24-desalkylsterols are present and to what extent We also have examined the alkane and alkanol content

RESULTS AND DISCUSSION

The lipids are discussed in the order of their elution from an alumina column

Hydrocarbons

The hydrocarbon fraction showed three main GLC peaks isothermally on XE-60 with RR_t 0 19, 0 25 and 0 40 The compounds were examined more carefully with a temperature program from 175° to 235° Based on a comparison with authentic standards, the principal hydrocarbons were identified as the C_{27}, C_{29}, and C_{31} *n*-alkanes together with small amounts of other chain lengths The complete list and relative amounts were C_{27} (13%), C_{28} (2%), C_{29} (43%), C_{30} (2%), C_{31} (30%), C_{32} (2%) and C_{33} (7%) The mass spectrum of the mixture showed ions at *m/z* (relative intensity) 464 (4), 450 (3), 436 (29), 422 (5), 408 (100), 394 (14), 380 (84), 366 (22) The ^{1}H NMR spectrum of the mixture was consistent with the assigned structures and showed the absence of branched-chain hydrocarbons

Fatty alcohols

Fatty alcohols were separated from the 4,4-dimethylsterols by differential solubility in hexane The fatty alcohols were precipitated essentially completely by solution of the mixture (with 4,4-dimethylsterols) in hot hexane followed by rapid cooling to *ca* 0° GLC analysis of the precipitate on XE-60 showed virtually a single peak with RR_t 0 68 On comparison with authentic *n*-alkanol standards on an SP-1000 column at 200°, the alkanol was identified as C_{28} (*n*-octacosanol) Mass spectral analysis showed an intense peak at *m/z* 392 $[M-H_2O]^+$, confirming the predominance of C_{28}, with smaller peaks at *m/z* 364 and 420 indicating trace amounts of the C_{26}- and C_{30}-alkanols

4,4-*Dimethylsterols*

After separation from the fatty alcohols, the 4,4-dimethylsterol fraction (hexane-soluble material) was chromatographed on TLC The material which moved with the rate of lanosterol was submitted to GLC analysis on XE-60 Two components were evident with RR_ts 1 70 and 1 96 The RR_ts of the two compounds corresponded well with those of authentic cycloartenol and 24-methylenecycloartanol The compounds were not further investigated but have been reported earlier by Bolger *et al* [8] to be present in another species of this genus, *C campbelli*

4-*Desmethylsterols*

The fourth material from the alumina column was the 4-desmethylsterol fraction On TLC this fraction moved with the rate of authentic cholesterol GLC analysis showed a peak with RR_t 1 42 After crystallization, the compound melted at 149° A UV spectrum indicated the absence of conjugated double bonds, the IR spectrum showed ν_{max} 890, 1650 (terminal methylene), 960 (*trans*-disubstituted double bond) and 802 cm^{-1} (trisubstituted double bond) Peaks appeared in the ^{1}H NMR spectrum at δ0 70 (*s*, C-18), 0 84 (*t*, C-29), 1 02 (*s*, C-19), 1 02 (*d*, C-21) and 1 65 (*s*, C-26) and in the mass spectrum at *m/z* (rel int) [fragment] 410 (13) $[M]^+$, 395 (4) $[M-Me]^+$, 381 (12) $[M-C_2H_5]^+$, 377 (4) $[M-Me-H_2O]^+$, 363 (12) $[M-C_2H_5-H_2O]^+$, 309 (11) $[M-C_6H_{11}-H_2O]^+$, 300 (40) $[M-C_7H_{11}O]^+$, 273 (19) [M – side chain]$^+$, 272 (40), 271 (100), 255 (76) [M – side chain – H_2O]$^+$, 253 (21), 231 (10), 229 (14), 227 (11), 215 (22) and 213 (34). Mass spectral analysis also indicated a few per cent of a contaminant with $[M]^+$ at *m/z* 412, which was probably the 22-dihydroderivative of the major sterol The IR, mass and ^{1}H NMR spectra of the dominant 4-desmethylsterol were identical with those obtained by Bolger *et al* [9] and Nes *et al* [2] for 24β-ethylcholesta-5,22*E*,25(27)-trien-3β-ol [25(27)-dehydroponiferasterol] The trace component with $[M]^+$ at *m/z* 412 was probably 24β-ethylcholesta-5,25(27)-dien-3β-ol (clerosterol) which is the dominant sterol in *C infortunatum* [7] and apparently in some other *Clerodendrum* species [11, 13] No evidence was obtained for any other 4-desmethylsterol

The presence of $\Delta^{25(27)}$-24β-ethylsterols in *Clerodendrum* (including the species examined here) is consistent with a 24β-alkylation mechanism which proceeds via removal of a proton from C-27 and is engrained in the genus without the mechanism for formation of 24-methylsterols Also, different species within the genus seem to differ in their sterol composition only by the extent of dehydrogenation at C-22(23), since some species contain either mostly clerosterol or mostly 25(27)-dehydroponiferasterol The sterol pattern is remarkably divergent from that in 'main line' plants [1, 2] and actually appears to be closer to that in many algae [1] *Clerodendrum* thus seems to be less highly developed than the majority of angiosperms, perhaps having had a different evolutionary experience However, the isolation of 24β-ethylsterols from plants other than species of *Clerodendrum* eliminates use of these sterols as a chemotaxonomic marker of this genus

EXPERIMENTAL

Leaves and stems of the vine, *Clerodendrum splendens*, grown in a very large greenhouse at Longwood Gardens, Kennet Square, PA, were air-dried for 3 days The dried plant material (1 5 kg) was crushed and macerated with a Polytron and extracted with Me_2CO for 32 hr in a Soxhlet After removal of the solvent, the residue was saponified in refluxing 10% methanolic KOH for 90 min The neutral lipid fraction was extracted with Et_2O and chromatographed on Al_2O_3 (4% H_2O) with Et_2O graded into hexane The various fractions, *viz* hydrocarbon, fatty alcohol, 4,4-dimethylsterol and 4-desmethylsterol obtained in the order given were analysed by GLC on 1% XE-60 at 235° using an FID with He as carrier gas RR_ts were determined relative to cholesterol TLC was performed on silica gel plates using 10% Et_2O in C_6H_6 as the solvent system UV spectra were obtained in EtOH IR spectra were determined in KBr pellets ^{1}H NMR spectra were determined at 220 MHz for samples in $CDCl_3$ with TMS as int standard MS (EI, 70 eV) was performed by Morgan Schaffer of Montreal, Canada Mps were obtained on a Kofler hot stage Recrystallizations were from MeOH

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ON THE REPORTED OCCURRENCE OF A FUSICOCCIN CONJUGATE IN MAIZE COBS

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Key Word Index—*Zea mays*, Gramineae, cobs, fusicoccin, phytotoxin, plant growth substance

Abstract—The occurrence in immature maize cobs of a fusicoccin derivative, reported in 1980 by Russian workers, could not be confirmed Extraction and fractionation procedures were identical to those used by the Soviet authors and the analysis of the fractions mainly relied on a very sensitive and specific radioimmunoassay Possible reasons for these contradictory results are discussed

INTRODUCTION

The fungal metabolite fusicoccin (**1**) has many biological activities typical of plant hormones [1], but unlike them its effects are tissue non-specific and its metabolic stability in plant tissues is remarkably high [2] Furthermore, in contrast with what is expected for a phytohormone, its distribution in nature appears to be very restricted [3] Nonetheless, the number of plants responding *in vivo* to, and binding *in vitro* with fusicoccin is quite high [3], suggesting that one or more metabolites capable of interacting with fusicoccin-binding sites are present in higher plants [4] This has found experimental support from investigations which are still in progress in our group [3]

In 1980 Muromtzev *et al* [5] reported the occurrence in immature maize cobs of a fusicoccin-like compound and proposed that fusicoccins are a new type of phytohormone Thus, for the first time a fusicoccin was detected in