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ALKALOIDS FROM THE ROOT BARK OF *ZANTHOXYLUM MYRIACANTHUM*

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Key Word Index—*Zanthoxylum myriacanthum*; Rutaceae; benzophenanthridine alkaloids; nitidine; dihydronitidine.

Plant Zanthoxylum myriacanthum Wall. *ex* Hook. f. [1] (Voucher: P. G. Waterman 530 deposited at the Herbarium of the Royal Botanic Garden, Edinburgh). *Source.* The root bark was collected from trees growing beside the Genting Highlands road, Pahang State, Malaysia, between the 10 and 12 km signs. *Previous work.* TLC examination of the bark of material collected in Hong Kong revealed the presence of several alkaloids [2]. *Plant part examined.* Root bark.

Present work. Root bark (150 g) was extracted in a Soxhlet apparatus successively with petrol (bp 40–60°), $CHCl_3$ and MeOH. Column chromatography of the petrol conc over Si gel, eluting with hexane, gave sitosterol (8 mg) mp 138° identical in all respects (IR, TLC, mmp) with an authentic sample. Further elution with EtOAc gave dihydronitidine (23 mg) mp 209° from MeOH (lit. [3] 208–211°) M^+ 349·1307; $C_{21}H_{19}NO_4$ requires 349·1314. The sample was identical in all respects (UV, IR, MS, TLC, mmp) with synthetic dihydronitidine produced by the reduction of nitidine with $NaBH_4$.

On shaking with 1 N HCl the $CHCl_3$ concentrate gave a yellow ppt which was recrystallized from EtOH–HNO_3 to yield nitidine nitrate (53 mg) mp 239°, identical in all respects (UV, IR, TLC, mmp) with an authentic sample.

Trace amounts of the quaternary alkaloids magnoflorine and tembetarine were detected by previously described TLC procedures [4] in the partially purified MeOH extract.

Biological significance. The restricted range of alkaloids recorded from *Z. myriacanthum*, with nitidine predominating, is similar to that found in *Z. nitidum* DC [3] but unlike that of *Z. ailanthoides* Sieb. and Zucc. [5,6] to which it is supposedly closely allied [7]. The absence of the pentacylcic triterpene lupeol, noted previously [2], was confirmed in this study.

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CAROTENE EPOXIDES FROM THE *DELTA* TOMATO MUTANT

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; *Delta* tomato; γ-carotene-1′,2′-epoxide; δ-carotene-1′,2′-epoxide.

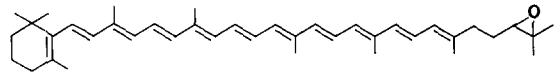
Although the carotenoid hydrocarbons of several tomato strains have been well characterized [1,2], very little is known about the oxygenated carotenoids present. Recently we have

isolated a series of carotenoids from normal red tomatoes, and identified them as epoxides of phytoene, phytofluene, ζ-carotene and lycopene [3,4]. It is well known that in the *Delta* tomato mutant δ-carotene (ε,ψ-carotene) replaces lycopene as the main pigment [2]. Examination of the more polar carotenoids present in an extract of *Delta* tomatoes has revealed the presence of two new carotenoids, identified as epoxides of δ-carotene and γ-carotene (β,ψ-carotene).

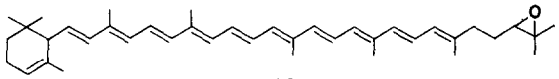
A chromatographic fraction was obtained with similar polarity to lycopene-1,2-epoxide isolated from normal red tomatoes. TLC on MgO–kieselgur G separated this fraction into four components, A–D. The most strongly adsorbed compound, A (R_f 0·1) was identical (absorption spectrum, MS, co-chromatography) to a sample of lycopene-1,2-epoxide (1,2-epoxy-1,2-dihydro-ψ,ψ-carotene) isolated from red tomatoes. Component B (R_f 0·25) was identical (absorption spectrum, MS, co-chromatography) to a sample of lycopene-5,6-epoxide (5,6-epoxy-5,6-dihydro-ψ,ψ-carotene) from red tomatoes. In particular losses of 69 m.u. in the MS confirmed the presence of the unsubstituted acyclic (lycopene) end group, and the ratio of the intensities of the M-92 and M-106 ions was characteristic of an acyclic carotenoid [5].

Compound C (R_f 0·55) had an absorption spectrum like that of γ-carotene but the polarity of an epoxide. The MS showed the molecular weight to be 552 ($C_{40}H_{56}O$) with major fragment ions at M-92 and M-106; the ratio of the intensities of these ions was characteristic of a monocyclic compound [5]. No losses of 56, 123 or 69 m.u. were observed showing that ε-ring and unsubstituted acyclic (lycopene) end groups were absent. The available data are consistent with the structure γ-carotene-1′,2′-epoxide (1′,2′-epoxy-1′,2′-dihydro-β,ψ-carotene, **1**).

Compound D (R_f 0·8) had an absorption spectrum like that of δ-carotene, the main carotenoid of *Delta* tomatoes. The MS showed the molecular weight to be 552 ($C_{40}H_{56}O$), and the ratio of the intensities of the M-92 and M-106 fragment ions was characteristic of a monocyclic structure. Losses of 56 and 123 m.u. from the parent ion and 56 m.u. from the M-92 and M-106 ions confirmed the presence of the unsubstituted ε-ring. No losses of 69 m.u. were observed, showing that no unsubstituted acyclic end group was present. The available data are consistent with the structure δ-carotene-1′,2′-epoxide (1′,2′-epoxy-1′,2′-dihydro-ε,ψ-carotene, **2**).



(1)



(2)

The amount of γ-carotene and δ-carotene epoxides present was of the same order as the amount of lycopene epoxides present in ordinary red tomatoes. Epoxidation of the isolated terminal double bond in the acyclic carotenoid end group thus appears to be a common reaction in strains of tomato and related fruit.

EXPERIMENTAL

Delta tomatoes were grown from seed in a greenhouse, and the fruits harvested when fully ripe. Lipid material was extracted with acetone and saponified by standard procedures [7] and the unsaponifiable material was chromatographed 2× on columns of neutral alumina (activity grade III) as previously described for ordinary red tomatoes [4]. The fraction eluted from the 2nd column with 10% Et_2O in petrol (light petrol, bp 40–60°) was chromatographed on thin layers of Si gel G with 10% Et_2O–petrol as developing solvent. The orange band (R_f 0·5) corresponding to a lycopene-1,2-epoxide marker was removed and eluted with Et_2O. TLC of this band on MgO–Kieselguhr G (1:1) with Me_2CO–C_6H_6–petrol (1:1:8) gave 4 bands, A–D, each of which was eluted and further purified by TLC on Si gel G with 5% Et_2O in petrol before determination of its absorption and MS. Compound A (1,2-epoxy-1,2-dihydro-ψ,ψ-carotene) had R_f 0·1, λ_{max} (petrol) at 443, 469, 500 nm and MS—M^+ 552 (100%, $C_{40}H_{56}O$) fragment ions at *m/e* 483 (2%, M-69, *m** 423; $483^2/552 = 422{\cdot}6$), 467 (1%, M-85), 460 (6%, M-92, toluene, *m** 383; $460^2/552 = 383{\cdot}4$), 446 (28%, M-106, xylene) and 377 (2%, M-106-69, *m** 319; $377^2/446 = 318{\cdot}7$). Compound B (5,6-epoxy-5,6-dihydro-ψ,ψ-carotene) had R_f 0·25, λ_{max} (petrol) at 428, 454, 486 nm. and MS—M^+ 552 (50%, $C_{40}H_{56}O$), fragment ions at *m/e* 483 (2%, M-69, *m** 423; $483^2/552 = 422{\cdot}6$), 460 (3%, M-92, *m** 383; $460^2/552 = 383{\cdot}4$), 446 (12%, M-106), 391 (1% M-92-69, *m** 332; $391^2/460 = 332{\cdot}4$), 377 (4%, M-106-69, *m** 319; $377^2/446 = 318{\cdot}7$) and 69 (100%). Compound C (1′,2′-epoxy-1′,2′-dihydro-β,ψ-carotene) had R_f 0·55, λ_{max} (petrol) at 433, 461, 492 nm and MS ions at *m/e* 552 (100%, M^+, $C_{40}H_{56}O$), 460 (4%, M-92, *m** 383; $460^2/552 = 383{\cdot}4$) and 446 (8%, M-106). Compound D (1′,2′-epoxy-1′,2′-dihydro-ε,ψ-carotene) had R_f 0·8, λ_{max} (petrol) at 429, 457, 488 nm and MS ions at *m/e* 552 (100%, M^+, $C_{40}H_{56}O$), 496 (0·5%, M-56), 460 (8%, M-92, *m** 383; $460^2/552 = 383{\cdot}4$), 446 (10%, M-106), 429 (2·5%, M-123, *m** 333·5; $429^2/552 = 333{\cdot}5$), 404 (1·5%, M-92-56; *m** 355; $404^2/460 = 354{\cdot}8$) and 390 (3%, M-106-56, *m** 341; $390^2/446 = 341{\cdot}0$). Spectra (absorption and mass) were obtained as previously described [4].

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MORETENOL AND OTHER CONSTITUENTS OF *CELTIS LAEVIGATA*

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Key Word Index—*Celtis laevigata*; Ulmaceae; *n*-alkanes; fatty acids; moretenol; sitosterol; stigmasterol.

Plant. Celtis laevigata Willd. *Source.* Montgomery, Alabama, U.S.A. *Use.* Source of Wood. *Previous work.* Cellulose and α-cellulose[1]. *Present work.* The dried, ground leaves (3·2 kg) were extracted by percolation with EtOH. After removal of the solvent *in vacuo* at 40°, the residue (321 g) was partitioned between 2% HCl and $CHCl_3$ to give basic (6 g) and non-basic (205 g) fractions. The non-basic fraction was fractionated by standard methods into neutral (179 g), acidic (9 g), and phenolic (11 g) fractions.

Neutral fraction. Chromatography over silicic acid and elution with light petrol. gave an alkane fraction which crystallized from EtOAc (15 mg); mp 63–65°; ν_{max}^{KBr} cm^{-1}: 2940, 2870, 1470, 1380, 730 and 720. GLC on a 160 cm column of 0·8% OV-17 on Gas Chrom Q (80–100 mesh) showed the mixture to be composed primarily of C_{25} to C_{35} *n*-alkanes; C_{25} (1%), C_{26} (2), C_{27} (10), C_{28} (11), C_{29} (20), C_{30} (12), C_{31} (22), C_{32} (5), C_{33} (5), C_{34} (1), C_{35} (1). The identity was confirmed by GC–MS. Elution with light petrol–$CHCl_3$ (1:3) gave a fatty acid fraction which crystallized from MeOH (350 mg) mp 77–78°; ν_{max}^{KBr} cm^{-1}: 2940, 2860, 1700, 1460, 1430, 1300, 930, 730, 720 and 680. GLC of the methyl esters on a 160 cm column of 0·8% OV-17 on Gas Chrom Q (80–100 mesh) showed the mixture to be composed of C_{24} (7%), C_{25} (6), C_{26} (29), C_{27} (5), C_{28} (38), C_{29} (3) and C_{30} (12) saturated straight fatty acids. The identity was confirmed by GC–MS.

Elution with light petrol–$CHCl_3$ (1:5) afforded a fraction which was re-chromatographed over silicic acid. Elution with C_6H_6–$CHCl_3$ (3:2) and subsequent preparative TLC on Si gel PF_{254} ($CHCl_3$, R_f 0·3) gave moretenol (60 mg) mp 225–226° (Abs. EtOH) (lit. [2] 236° [$CHCl_3$–MeOH]); $[\alpha]_D^{27}$ + 26·3° ($CHCl_3$, *c* 0·9) (lit. [2] + 27° [*c* 2·3]; λ_{max}^{EtOH} nm (log e): 205 (3·57) (lit. [2] 210 [2·73]); ν_{max}^{KBr} cm^{-1}: 3670, 3350, 3200, 3080, 2940, 2860, 1640, 1440, 1390, 1380, 1045, 990 and 890; MS M^+ *m/e* 426 (38%), 411 (14), 393 (5), 207 (41), 189 (100), 135 (37); $\delta_{60\,MHz}^{CDCl_3}$ 0·68 (Me, 3H, *s*), 0·75 (Me, 3H, *s*), 0·82 (me, 3H, *s*), 0·95 (2 × Me, 6H, *s*), 1·30 (Me, 3H, *d*), 1·60 (Me, 3H, *d*), 3·20 (CH–OH, 1H, *m*) and 4·70 (C = CH_2, 2H, *s*). Treatment with Ac_2O–C_5H_5N gave *O*-acetylmoretenol mp 266–268° (Light Petrol.) (Lit. [2] 283–285° [Et_2O–MeOH]); $[\alpha]_D^{27}$ + 24·0° $CHCl_3$, *c* 0·9) (lit. [2] + 24° [*c* 1·6]); ν_{max}^{KBr} cm^{-1}: 3080, 2940, 2860, 1725, 1640, 1440, 1390, 1380, 1250, 1025, 1005, 990, 980, and 890; MS M^+ *m/e* 468 (38%), 453 (9), 408 (12), 393 (12), 249 (8), 203 (14), 189 (100). Direct comparison (mp, mmp, Sp. Rotn., IR, MS) with an authentic sample of *O*-acetylmoretenol confirmed the identity. To our knowledge, this is the second report of the

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