H, 10·5. Calc. for $C_{30}H_{48}O_4C$, 76·2; H, 10·2%); diacetate with Py–Ac_2O at room temp, mp 183–185°, PMR ($CDCl_3$, δ) 0·87 (*s*, 9H), 1·00 (*s*, 6H), 1·17 (*s*, 3H), 1·27 (*s*, 3H), 2·1 (*s*, 6H), 5·35 (*m*, 1H); methyl ester diacetate, mp 170–171°, $[\alpha]_D$ +62·5 (*c*, 0·8 $CHCl_3$). These data indicated that (j) is a dihydroxy triterpene carboxylic acid with one hydroxyl and carboxyl in D/E rings (*m/e* 264) and one hydroxyl in A/B rings (*m/e* 207). The base peak in the mass spectrum at *m/e* 219 arises by a loss of C-28 function of Δ^2 oleananes. However, the facile alkaline hydrolysis (10% methanolic KOH, 5 hr) of methyl ester diacetate to give back the parent acid and absence of any methyl signal upfield from δ0·775 in PMR spectrum of acetate [6] ruled out its location on C-28, but the data were consistent with a C-29 or C-30 carboxyl function [7,8]. Of the two hydroxyls, both of which are secondary, one is likely to be at 3β-. In view of the easy acylation, the other hydroxyl could not be located at 15α-, β- or 16α-. Since MS data rule out its location in E ring, the only available position is 16β- and hence (j) could be 3β, 16β- dihydroxyolean-12-en-29- or 30-oic acid.

The remaining compounds identified were (k) isoliquiritigenin (25 mg), from C_6H_6–EtOAc (1:1) fraction followed by preparative TLC obtained as yellow prisms (EtOAc), mp 198–199°, deep brown colour with alc. $FeCl_3$, negative Shinoda and Wolfrom tests, λ_{max} (MeOH) 368 nm., confirmed by direct comparison (mmp, co-TLC and co-IR); (l) liquiritigenin (15 mg) as colourless rectangular plates (MeOH), mp 204–206°, negative ferric reaction and positive Shinoda's test, λ_{max} (MeOH) 230 (inf.), 276 nm, identical with cyclisation product of (k) with alc. HCl; (m) sitosterol-β-D-glucoside (120 mg) from C_6H_6–EtOAc (2:8) fractions as colourless rods (pyridine) mp 302–307° (d), positive LB, TNM, Molisch tests, acetate, mp 168–170°, $[\alpha]_D$ −30·7° (*c*, 0·825, $CHCl_3$) and finally (n) sucrose (250 mg).

The alcohol extract did not give any isolable material.

It would appear that the bark of *D. sericea* is specially rich in terpenoids as compared to other *Dalbergia* species.

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β-CITRAURINENE, A NEW C_{30}-CITRUS CAROTENOID*

URS LEUENBERGER and IVAN STEWART

Agricultural Research and Education Center, University of Florida, IFAS, Lake Alfred, FL 33850, U.S.A.

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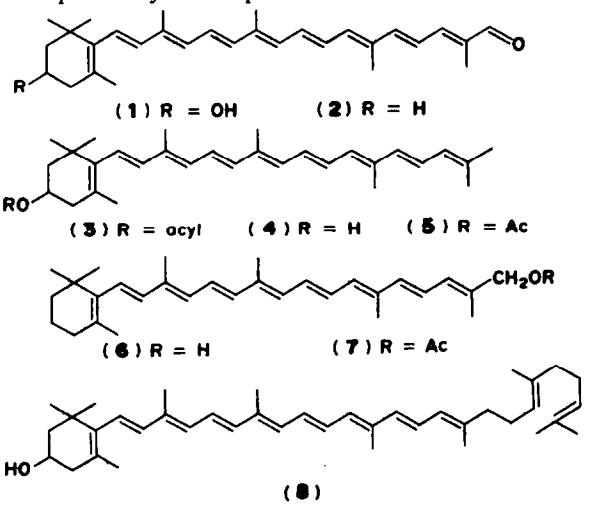
Key Word Index—*Citrus*; Rutaceae; carotenoid; β-citraurinene; 8'-apo-β-caroten-3-ol.

The pigment contributing mainly to the reddish and deep orange color in the peel of mandarins, oranges and many citrus hybrids is β-citraurin (3-hydroxy-8'-apo-β-caroten-8'-al) (**1**) [1,2]. The biosynthesis of β-citraurin in citrus peel was found to be greatly increased when harvested fruit was stored in an atmosphere containing up to 10 ppm ethylene and at temperatures below 30°. Simultaneously, 8'-apo-β-caroten-8'-al (**2**) increased to a lesser extent [2,3]. However, an unknown carotenoid was also found to greatly increase along with the two above mentioned C_{30}-pigments. Previously, it was thought to be cryptoxanthin, but later was found to be a mixture of cryptoxanthin and an unknown, with the unknown predominating in some cultivars. This unknown compound has been isolated from a citrus hybrid, Robinson (Orlando tangelo × Clementine), and identified by means of visible, IR, MS, NMR spectra and by chromatographic and chemical properties to be β-citraurinene (8'-apo-β-caroten-3-ol (**4**), a compound not believed to have previously been reported.



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Preliminary studies using HPLC [4] indicated that the peel of several citrus cultivars contained an unknown carotenoid with a chromophore similar to β-zeacarotene. The pigment occurred in the peel as an ester (3) as indicated from changes in R_f-values by TLC following saponification. The unknown carotenoid was assumed to be a monool since it separated with that group of compounds in liquid–liquid extraction and on chromatographic systems. This alcohol was difficult to separate from a mixture with cryptoxanthin and was also extremely unstable following crystallization; the crystalline product lost its color within hours. The acetylated compound was found to be more stable and this ester was used for most of the characterizations.

Visible spectra of the natural ester (3), the alcohol (4) and its acetate (5) were typical of a β,ψ-chromophore with λ_{max} 403, 425 and 451 nm in *n*-hexane. The molecular ion of the alcohol (4) was observed at *m/e* 418·3210 (Calc. for $C_{30}H_{42}O$:418·3235) besides the loss of water at *m/e* 400·3144 (Calc. for $C_{30}H_{40}$:400·3129). The acetate (5) exhibited a MW of 460·3361 (Calc. for $C_{32}H_{44}O_2$:460·3340) and the loss of HOAc at *m/e* 400·3279. From these MS data, a compound with a skeleton as (8) (3-hydroxy-β-zeacarotene) was eliminated, though the visible spectra were similar [5].

The hydroxyl group in β-citraurinene (4) was detected in the IR-spectrum at 3300 and 1045 cm^{-1} as were the ester signals of the acetate (5) at 1735 and 1255 cm^{-1}.

The nature of the hydroxyl group was investigated by several means. A tertiary alcohol as well as an OH-group at C-2 was excluded by the smooth and complete acetylation with Ac_2O in pyridine in less than 2 hr, followed by complete saponification within 30 min [6]. The OH-group was not found to be allylic to the main chromophore, i.e. C-4 or C-8′. This was indicated when an attempted oxidation with *p*-chloranil and treatment with acidified $CHCl_3$ did not give products with elongated chromophores [7,8]. When 6 was similarly treated with *p*-chloranil, the aldehyde (2) was readily formed within 4 hr. Furthermore, 5 and 7 could be separated with HPLC.

Final proof of β-citraurinene as 4 and its distinction from 8′-apo-β-caroten-8′-ol (6) was obtained from NMR-data. Although β-citraurinene (4) was not sufficiently stable to give a clear spectrum, there were signals at 1·07 (geminal methyl groups of a substituted β-ionone ring), 1·73 (methyl group at C-5), 1·96 (in-chain methyl groups) and 1·83 ppm (terminal methyl groups). As expected, the acetate (5) exhibited a doublet for the 1,1-Me groups at 1·08 and 1·12 ppm with an integral for 6 protons compared with a singlet peak at 1·04 ppm for structure 7.

The proton of C-3 in β-citraurinene acetate (5) showed a signal at 5·05 ppm confirming an ester at this carbon, whereas compound 7 had no such signal, but a typical peak at 4·58 ppm for the CH_2 group at C-8′. The two methyl groups at C-8′ for 5 could be detected at 1·84 ppm as a singlet with 6 protons, whereas the spectrum of 7 gave an expected singlet at 1·86 ppm for a single methyl group in the same position. The ester methyl group of 5 appeared at 2·05 ppm compared with the similar peak of 7 at 2·10 ppm. The total integral of the protons of compound 5 also agreed with the amount of hydrogen found with the MS ruling out a longer molecule like 8.

Preliminary examination indicated that β-citraurinene is present in many citrus cultivars and occurs as a major pigment in some. Although the amount in the peel varies depending on maturity and other factors, three samples of Robinson peel collected in February, contained 4·1 μg/cm² ± 0·7 of β-citraurinene. For comparison, one sample of the peel was found to contain 2·4 μg/cm² of cryptoxanthin. The value given are those of the acetates.

Carotenoids having 8′-apo structure are not common in Nature; most of those known, occur in the peel of citrus fruits. While the present work was underway, Gross *et al.* [9] reported finding a C_{30}-carotenoid in citrus juice, 3-hydroxy-5, 8-epoxi-5,8-dihydro-8′-apo-β-caroten-8′-al. Previously Yokoyama *et al.* [10,11] described carotenoids in Sinton citrangequat and grapefruit with visible spectra similar to β-citraurinene, but they did not determine their structure.

Taylor and Davies [12,13] recently reported on a series of C_{30}-carotenoids from bacteria and postulated a biosynthetic pathway. In citrus it has been proposed that C_{30}-carotenoids are degradation products of zeaxanthin [14]. However, when considering the new hydrocarbon endgroup in β-citraurinene, they may well have a metabolic pathway different from that of the C_{40} compounds.

EXPERIMENTAL

Source of material. Robinson fruit were collected from groves in Central Florida during December and January.

Isolation. Details of extraction with MeOH and $C_2H_4Cl_2$ (1:1) are described elsewhere [1]. Oil was removed in vacuo and the saponified mixture partitioned between *n*-hexane and 90% MeOH. Hydrocarbons and monohydroxy compounds were collected in the epiphase. Preliminary separation of pigments in this layer was made on a column filled with MgO–cellite (1:1) activated at 240° overnight. The solvent mixture consisted of starting with *n*-hexane and using increasing amounts of $C_2H_4Cl_2$. Following chromatogram development, absorbent was pushed from the column. The portion containing compound 4, which was slightly more polar than cryptoxanthin, was eluted with MeOH. This fraction was acetylated in Py with Ac_2O. Additional purification of β-citraurinene acetate was made on a column packed with Al_2O_3 act. II–III. Starting with a solvent mixture of 10% C_6H_6 in *n*-hexane, fractions were eluted, collected and monitored by visible absorption spectra. By this means the *trans* isomer was separated from the *cis* forms. *trans* β-Citraurinene acetate was crystallized from C_6H_6–MeOH yielding 15 mg from peel of about 80 kg of fruit.

TLC studies. TLC studies were carried out with precoated Si gel plates (Merck Art. 5539/0001 and 5765) and C_6H_6–*n*-hexane–EtOH (25:25:1).

HPLC. Separations were made by modifications of previously published procedures [4]. Esters were resolved on a 3 mm × 23 cm col, packed with MgO, and using 5% MeCOEt in *n*-hexane.

β-citraurinene 4. Small orange needle-bundles, λ_{max} (*n*-hexane) 403, 425 and 451 nm; ν_{max} (KBr) 3300 (bonded OH), 3035–2860 (CH), 1455 (CH_2, Me), 1395–1350 (Me), 1045 (OH), 960 (*trans*-CH=CH-)cm^{-1}; NMR (100 MHz, $CDCl_3$, TMS):δ 1·96 *s* (in-chain Me), 1·83 *s* (Me, C-8′), 1·73 *s* (Me, C-5), 1·07 (Me, C-1).

β-citraurinene acetate 5. Crystals similar to 4. λ_{max} (*n*-hexane) 403, 425 ($E^{1\%}_{1cm}$ = 2685) and 451 nm; ν_{max} (KBr) 2995–2860 (CH), 1737 (C=O), 1460 (CH_2, Me), 1370 (Me), 1255 (C–O), 1030, 973 and 960 (*trans*-CH=CH-)cm^{-1}; NMR (100 MHz, $CDCl_3$, TMS):δ 6·7–5·9 (12 H, olefinic), *ca* 5·05 (1 H, C-3), 2·36 and 2·23 (CH_2, C-4), 2·05 *s* (Me of acetate), 1·97 *s* (3 × Me, C-9, 13, 13′), 1·84 *s* (2 × Me, C-8′ and 19′), 1·74 *s* (Me, C-5), 1·55 (CH_2, C-2), 1·12 *s* and 1·08 *s* (2 × Me, C-1); isotope ratio M^+:M + 1:M + 2 = 100:43:11, Calc. 100:37:7), 400 (M–60), 368 (M–92), 308 (M–92–60).

8'-apo-β-caroten-8'-ol acetate 7. Made by reduction of synthetic 8'-apo-β-caroten-8'-al (2) with $LiAlH_4$ [15,16], followed by acetylation with Ac_2O in Py [15,17]. Orange–reddish plates. λ_{max} (*n*-hexane): 402, 425 ($E^{1\%}_{1cm}$ = 2665 as found by Rüegg *et al.* [18]) and 450 nm; ν_{max} (KBr): 2985–2860 (CH), 1745 (C=O), 1460 (CH_2, Me), 1365 (Me), 1240 (C–O), 1040, 980 and 965 cm^{-1} (*trans*-CH=CH-); NMR (100 MHz, $CDCl_3$, TMS) δ 6·8–6·11 (12 H olefinic), 4·58 *s* (CH_2, C–8'), 2·10 *s* (Me of acetate), 2·04 (CH_2, C–2), 1·99 *s* (3 × Me, C–9, 13, 13'), 1·86 *s* (Me, C–9'), 1·73 *s* (Me, C–5), *ca* 1·6 and 1·45 (protons of C–2 and C–3), 1·53 *s* (impurity: H_2O), 1·04 (2 × Me, C–1); similar NMR-values are given in [19].

Oxidation with p-chloranil [8]. A mixture of *ca* 1 mg of **4** or **6** in 0·5 ml C_6H_6 and 1 mg *p*-chloranil were let stand for 15 hr under N_2 in the dark at room temp. Synthetic alcohol (**6**) was converted to the corresponding aldehyde, whereas β-citraurinene (**4**) yielded mainly the starting compound with only traces of other products.

Treatment with acidified chloroform [20,21]. To *ca* 1 mg of β-citraurinene (**4**) was added 2 ml of *ca* 0·05 N HCl in $CHCl_3$. No color change was observed during 30 min. reaction time in the dark. Pigments were transferred to Et_2O upon admixture of aq bicarbonate soln. Only polar decomposition products (*ca* 10%), traces of a non-polar compound (λ_{max}: 401 (*sh*), 423 and 446 nm in *n*-hexane) and mainly unchanged β-citraurinene (**4**) (*ca* 85%) were detected in the reaction mixture by TLC examination. 8'-apo-β-caroten-8'-ol (**6**) yielded in a similar reaction mainly a non-polar product with λ_{max} 430 nm in *n*-hexane, without fine structure.

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AURENTIACIN, A NEW CHALCONE FROM *DIDYMOCARPUS AURENTIACA*

NARAYAN ADITYACHAUDHURY, ASIT K. DAS, ASHIM CHOUDHURY and PANNA L. DASKANUNGO
Department of Agricultural Chemistry & Soil Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswa Vidyalaya, West Bengal, India

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Key Word Index—*Didymocarpus aurentiaca*; Gesneriaceae; aurentiacin; 2'-hydroxy-4',6'-dimethoxy-3'-methylchalcone.

Phytochemical investigation of *Didymocarpus pedicellata* has revealed the presence of a number of chalcones, quinochalcones and flavanones [1, 2]. These results prompted us to examine another species, *D. aurentiaca*, growing in the Darjeeling area, West Bengal. A new chalcone, aurentiacin, has been isolated from this plant and its structure-elucidation is described here.

It was obtained as an orange coloured crystalline compound, $[\alpha]_D \pm 0°$ ($CHCl_3$), $C_{18}H_{18}O_4$ (M^+ 298). Colour reactions indicated it to be chalcone. Functional group analysis revealed the presence of two OMe (two 3H singlets at 3·95δ and 4·0δ), one aromatic – Me (3H singlet at 2·06δ), a conjugated >C=O (ν^{KBr}_{max} 1620 cm^{-1}), a chelated –OH (ν^{KBr}_{max} 3200 cm^{-1}; 1H singlet 14·06δ, exchangeable with D_2O, brown colour with $FeCl_3$) and a complex aromatic substitution pattern (ν^{KBr}_{max} 1600, 1550, 1125, 790, 745 cm^{-1}) with an unsubstituted benzene ring [2] (ν^{KBr}_{max} 700 cm^{-1}). The presence of six aromatic protons was also discernible in the NMR spectrum. The NMR spectrum also showed 2 *trans*-olefinic protons at 7·7δ as a 2H—singlet providing evidence for a chalcone system [1,3]. The mass spectrum showed peaks characteristics of chalcones [4]. The unsubstituted nature of the B-ring of aurentiacin was readily apparent from the appearance of 2 prominent peaks at *m/e* 221 (M^+–77; $M^+ - C_6H_5$) and *m/e* 195 (M^+ – 103; $M^+ - C_6H_5$ – CH