ators on the market, coupled with the expanding investigation of O_3 water purification, has made ozonation available to most laboratories.
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Isolation and Identification of β-Citraurol, a C_{30} Carotenoid in Citrus[1]

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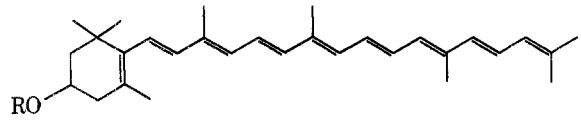
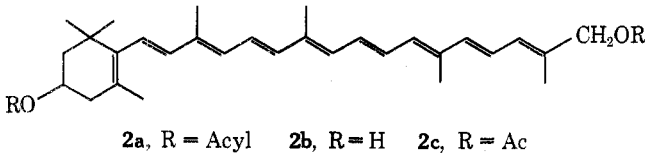
Received July 10, 1975

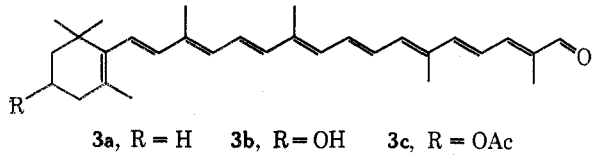
Reported occurrences of 8′-apocarotenoids in nature are not common. From a known total of 311 naturally occurring carotenoids Straub[2,3] lists only four C-30 compounds. Two of these, 8′-apo-β-caroten-8′-al (**3a**) and β-citraurin (**3b**), are found in citrus peel. The latter pigment is the major contributor to the red color of tangerines and the bright color of oranges.[4,5] More recently, 3-hydroxy-5,8-epoxy-5,8-dihydro-8′-apo-β-caroten-8′-al was found in orange juice[6] and β-citraurinene (8′-apo-β-caroten-3-ol, **1a**) was identified as a major pigment in citrus peel.[7] Also recently, Taylor and Davies[8,9] reported a group of acyclic C-30 carotenoids from bacteria.



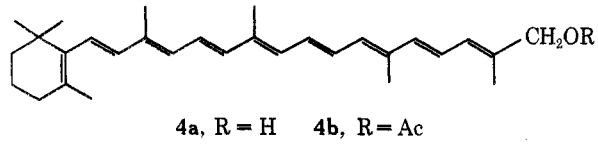
1a, R = H **1b**, R = Ac



2a, R = Acyl **2b**, R = H **2c**, R = Ac



3a, R = H **3b**, R = OH **3c**, R = OAc



4a, R = H **4b**, R = Ac

In this paper, we describe the isolation and structure elucidation of a new C_{30} carotenoid, β-citraurol, from peel of the citrus hybrid Robinson (Orlando tangelo × Clementine mandarin). This carotenoid with the structure of 8′-apo-β-caroten-3,8′-diol (**2b**) is not believed to have previously been reported to occur in nature. Curl reported the synthesis of β-citraurol from β-citraurin.[10]

β-Citraurol (**2b**) occurs as a diester in the peel as determined by a significant change in R_f on TLC following saponification. Acetylation of the carotenoid indicated two hydroxyl groups, yielding a monoester as an intermediate. The natural diester (**2a**), the diol (**2b**), and the diacetate (**2c**) showed similar visible spectra typical for a β,ψ chromophore with λ_{max} 403, 425, and 450 nm in *n*-hexane. There was no shift in the visible spectrum after treatment with HCl in EtOH[11] and a color test with HCl on TLC was negative. These tests along with a failure to reduce the oxygen with lithium aluminum hydride indicated the absence of an epoxide or a furanoid oxide.

Most of the studies on β-citraurol reported in this paper were made on the diacetate ester. The ester was more easily isolated than the alcohol and previous work with similar compounds would suggest that the esterified form is more stable.[7] β-Citraurol diacetate (**2c**) exhibited a molecular ion at *m/e* 518. Fragments due to the loss of acetic acid at *m/e* 458 (M − 60) and 398 (M − 120) confirmed the presence of two hydroxyl groups. Typical ester bands were exhibited in the infrared spectrum at 1740 and 1245 cm^{-1}.

The positions of the OH groups were investigated by several means. Smooth saponification and acetylation reactions ruled out a tertiary alcohol as well as a hydroxyl group in position C-2.[12] Oxidation with *p*-chloranil yielded a reddish compound with chromatographic and spectral properties identical with those of β-citraurin (**3b**) indicating one allylic hydroxyl group.

The above mentioned structural features were ultimately confirmed by the NMR data. The doublet of the geminal methyl groups at 1.08 and 1.12 ppm as well as the broad signal of the single proton at C-3 at ca. 5.05 ppm point to a secondary ester configuration of a 3-hydroxy-β-end group ring. A singlet at 1.98 ppm was associated with the three in-chain methyl groups, while the end-of-chain methyl yielded a singlet at 1.85 ppm. These signals together with the singlet of two protons at 4.58 ppm demonstrated an ester of a primary alcohol group at C-8′. Finally, the structure was confirmed when the diacetate of β-citraurol was synthesized from β-citraurin and the synthetic product was found to have identical chromatographic, spectroscopic, and chemical properties with the diacetate made from the natural occurring diol.

The finding of β-citraurol brings the known 8′-apocarotenoids to seven, with five of these found in citrus fruit. The presence of this number of similar compounds suggests that the biosynthesis of β-citraurin may not be a degradation product of C_{40} compounds as proposed[13] but rather it may form through a new pathway for C_{30} compounds.

A novel fragmentation of β-citraurol diacetate in the mass spectrometer was observed with both the natural and synthetic compounds. It is well known in carotenoid chemistry that acetic acid esters of nonallylic hydroxy carotenoids have a fragment M − 60 (M − acetic acid). The allylic ester of β-citraurol at C-8′ showed not only a loss of acetic acid but also two significant fragments, one at M − 44, loss of C_2H_4O, and a second fragment at M − 58, loss of $C_2H_2O_2$. The intensity of *m/e* 474 varied from 1.8 to 77% compared with the molecular ion and *m/e* 460 varied from 6.3 to 108% depending on the probe temperature.

The fragments *m/e* 474 and 460 have the same compositions as compounds **3c** and **1b**, respectively. Therefore, the decomposition of **2c** into **3c** and **1b** is not excluded. The relatively high temperatures required (>200°C) and the variable fragment intensities suggest thermal rather than electron-impact induced reactions. Compound **1b** does not exhibit a similar fragmentation, but gives only the M − 60 peak. However, 8′-apo-β-caroten-8′-ol acetate (**4b**) also gives fragments of M − 44 and M − 58 as found with **2c**. This would suggest that these two fragments are characteristic for the allylic end-of-chain acetate ester. Gross et al.[14] report for a similar allylic end-of-chain ester of 5,8-epoxy-5,8-dihydro-10′-apo-β-caroten-3,10′-diol diacetate a frag-

ment M − 42 (ketene). We did not observe any M − 42 peaks from **2c** or **4b**.

Experimental Section

Isolation of β-Citraurol. Robinson fruits grown in Florida were collected during December and January. The peels were frozen and then extracted with dichloroethane–methanol (1:1) and $MgCO_3$ in a Waring blender. The filtered extract was dried, redissolved in ether, and saponified with 10% methanolic KOH. After washing and drying, the carotenoids were partitioned in hexane–methanol (90:10). A preliminary separation of the pigments in the methanol layer was made on a column filled with MgO–Celite (1:1) activated at 240°C overnight. The solvent mixture consisted of starting with hexane and using increasing amounts of dichloroethane. β-Citraurol was slightly less polar than zeaxanthin. The fraction containing **2b** was acetylated in pyridine with acetic anhydride. The β-citraurol acetate was purified by passing through a column packed with alumina Woelm W 200 basic acitivity II–III. Starting with a solvent mixture of 10% benzene in hexane, fractions were eluted, collected, and monitored by visible absorption spectra. By this means, the trans isomer was separated from the cis forms. The trans β-citraurol diacetate **2c** was crystallized from benzene–methanol yielding small, orange needles: λ_{max} (*n*-hexane) 403, 425, 450 nm; ir (KBr) 3040–2860 (CH), 1740 (C=O), 1445 (CH_2, CH_3), 1365 (CH_3), 1245 (CO–), 1030 and 970 cm^{-1} (trans CH=CH–) cm^{-1}; NMR (100 MHz, $CDCl_3$, Me_4Si) δ 6.7–6.1 (olefinic protons), ca. 5.05 (H of C-3), 4.58 s (CH_2 of C-8′), 2.36 and 2.24 (CH_2 of C-4), 2.10 s (CH_3 of acetate at C-8′), 2.06 s (CH_3 of acetate at C-3), 1.98 s (CH_3 at C-9, 13 and 13′), 1.85 (CH_3 at C-8′), 1.73 s (CH_3 at C-5), 1.52 s (impurity H_2O), 1.26 s (impurity), 1.12 and 1.08 (2 CH_3 at C-1), 0.89 and 0.84 ppm (impurities); mass spectrum M^+ 518.3430 (calcd for $C_{34}H_{46}O_4$, 518.3393); isotope ratio (M^+):(M + 1):(M + 2) 100:38:10 (calcd, 100:43:9), 474.3257 (M − 44 or M − C_2H_4O, calcd for $C_{32}H_{42}O_3$, 474.3133), 460.3336 (M − 58 or M − $C_2H_2O_2$, calcd for $C_{32}H_{44}O_2$, 460.3340), 458.3173 (M − 60 or M − $C_2H_4O_2$, calcd for $C_{32}H_{42}O_2$, 458.3184), 426 (M − 92), 414.2872 (M − 44 − 60, calcd for $C_{30}H_{38}O$, 414.2923), 400.3052 (M − 58 − 60, calcd for $C_{30}H_{40}$, 400.3129), 398 (M − 60 − 60), 366 (M − 60 − 92), 352 (M − 60 − 106), 263 (M − 60 − 195).

β-Citraurol Diacetate (2c). A solution of β-citraurin (**3a**) in tetrahydrofuran was reduced with lithium aluminum hydride,[15,16] followed by acetylation with acetic anhydride in pyridine[15,17] to obtain small, orange needles: λ_{max} (hexane) 404, 426, 452 nm; ir (KBr) 3040–2860 (CH), 1740 (C=O), 1445 (CH_2, CH_3), 1365 (CH_3), 1240 (CO–), 1025 and 965 cm^{-1} (trans CH=CH–); NMR (100 MHz, $CDCl_3$, Me_4Si) δ 6.9–6.1 (olefinic protons), ca. 5.05 (H of C-3), 4.56 s (CH_2 of C-8′), 2.36 and 2.24 (CH_2 of C-4), 2.09 s (CH_3 of acetate at C-8′), 2.05 s (CH_3 of acetate at C-3), 1.98 s (CH_3 at C-9, 13 and 13′), 1.86 s (CH_3 at C-8′), 1.74 s (CH_3 at C-5), 1.56 (impurity H_2O), 1.12 and 1.08 (2 CH_3 at C-1); mass spectrum M^+ 518.3426 (calcd for $C_{34}H_{46}O_4$, 518.3393), 474.3158 (M − 44 or M − C_2H_4O, calcd for $C_{32}H_{42}O_3$, 474.3133), 460.3355 (M − 58 or M − $C_2H_2O_2$, calcd for $C_{32}H_{44}O_2$, 460.3340), 458.3172 (M − 60 or M − $C_2H_4O_2$, calcd for $C_{32}H_{42}O_2$, 458.3184), 426 (M − 92), 414.3013 (M − 44 − 60, calcd for $C_{30}H_{38}O$, 414.2923), 400.3157 (M − 58 − 60, calcd for $C_{30}H_{40}$, 400.3129), 398 (M − 60 − 60), 366 (M − 60 − 92), 352 (M − 60 − 106).

8′-Apo-β-caroten-8′-ol Acetate (4b). This compound was prepared by reducing 8′-apo-β-caroten-8′-al with lithium aluminum hydride followed by acetylation with acetic anhydride in pyridine: mass spectrum M^+ 460.3340 (calcd for $C_{32}H_{44}O_2$, 460.3340), 416 (M − 44), 402.3260 (M − 58, calcd for $C_{30}H_{42}$, 402.3286), 400.3112 (M − 60, calcd for $C_{30}H_{40}$, 400.3129), 368.2725 (M − 92, calcd for $C_{25}H_{36}O_2$, 368.2714), 354.2537 (M − 106, calcd for $C_{24}H_{34}O_2$, 354.2557), 310.2604 (M − 58 − 92, calcd for $C_{23}H_{34}$, 310.2660), 308.2517 (M − 60 − 92, calcd for $C_{23}H_{32}$, 308.2503), 296.2462 (M − 58 − 106, calcd for $C_{22}H_{32}$, 296.2504), 294.2341 (M − 60 − 106, calcd for $C_{22}H_{30}$, 294.2347).

Oxidation of β-Citraurol (2b). β-Citraurol was dissolved in 0.5 ml of benzene and treated with *p*-chloranil (1 mg).[18] After 15 h there was almost complete conversion of **2b** to β-citraurin (**3b**). Characterization of **3b** was by visible spectrum in hexane and ethanol and by TLC using an authentic sample of β-citraurin (**3b**) for comparison.

Acknowledgment. We are grateful to Dr. W. Brey, Gainesville, Fla., for NMR spectra, and to Mr. G. Edwards, Lake Alfred, Fla., for the ir spectra. We are also indebted to Hoffmann-La Roche, Basel, for a sample of 8′-apo-β-caroten-8′-al.

Registry No.—**2b**, 57593-78-9; **2c**, 57593-79-0; **3a**, 650-69-1; **4b**, 38699-13-7.

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Mechanism of Ozonolysis. Triphenylphosphine Reduction of Methylisopropylethylene Ozonide-^{18}O

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Received October 30, 1975

When diisopropylethylene is ozonized in the presence of acetaldehyde-^{18}O, methylisopropylethylene ozonide-^{18}O is produced. The position of ^{18}O enrichment in the ozonide provides mechanistic information. In one study,[1] it was concluded that 68–77% of the ozonide formed by a pathway which placed the ^{18}O label at the peroxy site. This analysis included reduction of the ozonides by $LiAlH_4$ or $LiCH_3$ followed by mass spectrometry of the ethanol and isobutyl alcohol that was obtained.

In a subsequent report on the same compound,[2] it was argued that such pathways are considerably less important. An upper limit of 10% was estimated for them by comparing the mass spectral intensities of the ozonide parent ions and the ether fragment ions (loss of O_2). Most of the total ^{18}O enrichment in the parent ion was also found in the ether fragment but a small difference was reported. This difference could be attributed to a competing process producing peroxy ^{18}O incorporation such as the aldehyde interchange mechanism[3] or the enrichment of ozone by exchange with ^{18}O-aldehyde.[4] Other possible explanations are that small amounts of scrambling occurred between peroxide and ether oxygens upon fragmentation or that a systematic error occurred owing to the weak intensities of the mass peaks (perhaps arising from an undetected trace impurity contributing to the intensities).

In order to test the possibility of ^{18}O enrichment at the peroxide site more directly and clarify if there is as much as 10% competition from such pathways, several samples from our previous study[2] were treated with Ph_3P. This produced Ph_3PO which was analyzed for ^{18}O content. The basis of the method is the work of Lorenz and Park[5,6] and Carles