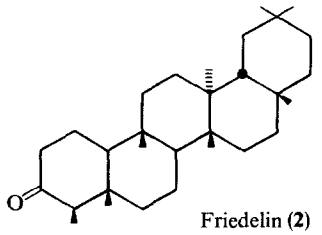
$C_{14}H_{22}O^+$ and $C_{15}H_{26}^+$ (1:5), 205 (40) $C_{14}H_{21}O,^+$ 191 (76) $C_{13}H_{19}O^+$ and $C_{14}H_{23}^+$ (1:10), 179 (44) $C_{12}H_{19}O^+$ and $C_{13}H_{23}^+$ (1:2), 178 (28), 177 (24), 149 (34), 137 (48) $C_9H_{13}O^+$ and $C_{10}H_{17}^+$ (1:8), 135 (40), 123 (60) $C_8H_{11}O^+$ and $C_9H_{15}^+$ (1:7), 121 (74), 109 (100) and 107 (48).

The peaks at *m/e* 302, 246 and 218, present in the MS of friedelin, (**2**) are not observed in that of filican-3-one. Other fragments as 341 (loss of E-ring) and 205 contain oxygen, unlike the fragments *m/e* 341 and 205 in friedelin. The fragments 341, 234 and 191 are more abundant in the mass spectrum of filican-3-one than in the spectrum of friedelin; the opposite is true for the 232 and 125 fragments [8–10]. These differences distinguish filican-3-one clearly from friedelin.



Friedelin (**2**)

IR spectrum ν_{max} (KBr-disc): 3400, 2900, 1705, 1455, 1380, 1260, 1200, 1070, 1005 and 800 cm^{-1}. NMR spectrum (100 MHz in $CDCl_3$): the most upfield signal is at δ0.71 ppm, like in friedelin it is assigned to 5-Me [11, 12]. Based on the integration, stereochemical considerations and the shifts observed for the Me groups upon increasing concentration of the shift reagent Eu $(FOD)_3$, the following assignments are made for the methyl signals: δ in ppm 0.71 (5-Me), 0.80 (17-Me), 0.83 (*d*, J = 6.5 Hz, 22-Me), 0.875 (*d*, J = 6.5 Hz, 4-Me), 0.89 (*d*, J = 6.5 Hz, 22-Me), 0.915 (6 H, 13-Me and 9-Me) and 0.99 (14-Me). In deuterobenzene the NMR spectrum showed the following shift for the methyl groups: δ in ppm, 0.68 (5-Me), 0.75 (17-Me), 0.835 (9-Me), 0.87 (13-Me), 0.901 (*d*, J = 6.5 Hz, 22-Me), 0.96 (*d*, J = 6.5 Hz, 22-Me), 0.975 (*d*, J = 6.5 Hz, 4-Me), and 1.01 (14-Me). This means that the 4-Me signal is shifted 0.10 ppm to the lower field, which is in accordance with the shift observed for friedelin, under the same circumstances [12]. This confirms the β-configuration of the 4-Me (= equatorial).

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CAROTENOID SYNTHESIS IN TURKISH LEMONS AND ORANGES AS INFLUENCED BY TRIETHYLAMINE DERIVATIVES

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Key Word Index—*Citrus*; Rutaceae; lemons; oranges; carotenoids; triethylamines; lycopene.

Abstract—Two triethylamines, 2-(4-chloro-phenylthio)-triethylamine (CPTA) and 4-chloro[β-(diethylamino)-ethyl]-benzoate (CDEB), were used to investigate C_{30} carotenoid biosynthesis in Turkish oranges and lemons. It was not possible to determine the pathway, since not all the intermediates from phytoene to the C_{30} compounds were observed. Lycopene and ζ-carotene, absent in controls, were identified in large amounts in certain CPTA- and CDEB-treated lemons and oranges. Furthermore there were varietal differences, since CDEB-treated Turkish oranges did not produce the increases in α- and β-carotene earlier observed in Californian *Citrus* fruits.

INTRODUCTION

Yokoyama *et al.* [1–3] have shown that CPTA affected carotenoid biosynthesis by increasing lycopene production whilst CDEB increased β-carotene over a certain period [4]. Since it is known that varietal differences played an important role in response to these compounds

(Yokoyama, personal communication) it was decided to study their effect on Turkish oranges and lemons to observe differences, if any, in these varieties.

RESULTS AND DISCUSSION

When we received the normal Turkish oranges and lemons they were yellow and pale yellow respectively. The flavedo of lemons treated with CPTA (5000 ppm) was red after 3 weeks and contained almost 10 times more total carotenoids compared to the controls. This was due mainly to lycopene accumulation which increased to 74% during this period with no corresponding increase in controls. In oranges, however, as well as increased lycopene synthesis there was also a marked increase in ζ-carotene as the oranges reddened after 3 weeks. The increase in certain carotenes suggests a decrease in other carotenoids. With lemons the decrease was mainly in β-apo-8′-carotenal and sintaxanthin (C_{30}), mutatochrome, zeaxanthin, violaxanthin and lutein (all C_{40} compounds). With oranges, however, the decrease involved mostly violaxanthin, cryptoxanthin and to a lesser extent lutein. This again suggests differences between different species of *Citrus* fruits. Yokoyama *et al.* [2] had concluded that in Californian oranges and grapefruits, the large accumulation of lycopene and γ-carotene suggests that CPTA acts either by inhibition of cyclases or by feedback inhibition; however, this may not be the complete picture (Yokoyama, personal communication).

Another triethylamine named CDEB has been used by Yokoyama *et al.* [5], who found that it not only increased lycopene but also α- and β-carotene. Some was made available to us and we studied its effects on Turkish oranges. As with CPTA, lycopene was obtained in large amounts (68%) in treated fruits, but not controls, at a concentration of 0.01 M CDEB. Although various concentrations were used over a period of 5 weeks, β-carotene synthesis never increased but in fact decreased, as did violaxanthin (from 54 to 8%). This may suggest that lycopene is the precursor of violaxanthin, but as there are several steps between lycopene and violaxanthin [6] this provides little help in studying the biosynthetic pathway of xanthophyll formation from carotenes. Another effect of CDEB on Turkish oranges was the production of *cis*-phytofluene, not identified in any other cases.

Our results with CDEB were very different to those of Poling *et al.* [5] who found increased α- and β-carotene syntheses. They found a rapid initial accumulation of lycopene which was later converted to β-carotene in Marsh white seedless grapefruit. This was not found with Turkish oranges, although our studies were carried out over a longer period of 5 weeks. It was hoped that all carotenoids leading to the C_{30} compounds would be identified in the treated oranges and lemons as has already been shown for red pepper [7], but this was not so in the case of Turkish oranges.

EXPERIMENTAL

Fruit samples. The Turkish lemons and oranges were harvested at different times from trees located in Izmir, Turkey. The fruits were then sent by air and took about 4 days to reach the laboratory.

Treatment with CPTA. The fruits were treated as previously described [2] but the wetting agent used was Triton X-100.

Treatment with CDEB. As previously described [5].

Extraction, isolation and quantitative determination of pigment. The carotenoids were isolated and separated as previously described [6]. Carotenoids having epoxy groups were characterised by the modified conc HCl–ether test [8]. The structural identity of individual carotenoids was established by comparison with authentic samples using various chromatographic methods [6, 7] and visible, UV and IR spectroscopy. The apo- compounds were further identified by reduction with $LiAlH_4$ when the corresponding mono-ol was obtained [9]. The concn of individual carotenoids was determined by measuring E_{max} and comparing it with known $E^{1\%}_{1\,cm}$ for pure pigments [10]. For those pigments for which $E^{1\%}_{1\,cm}$ were not known, λ_{max} was assumed to be 2500 [6] All results were calculated on a per g dry wt basis and each carotenoid was estimated as the percentage of total carotenoids.

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