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APOCAROTENOIDS FROM *COCHLOSPERMUM TINCTORIUM*

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Key Word Index—*Cochlospermum tinctorium*; Cochlospermaceae; apocarotenoids; cochloxanthin; dihydrocochloxanthin.

Abstract—Seven carotenoids have been isolated from *Cochlospermum tinctorium* by means of countercurrent chromatography and HPLC. The two major constituents were identified by spectroscopic methods (UV-VIS, IR, ^{1}H NMR, ^{13}C NMR and EIMS) as 6-hydroxy-8′-apo-ε-caroten-3-one-8′-oic acid (cochloxanthin) and 4,5-dihydro-6-hydroxy-8′-apo-ε-caroten-3-one-8′-oic acid (dihydrocochloxanthin).

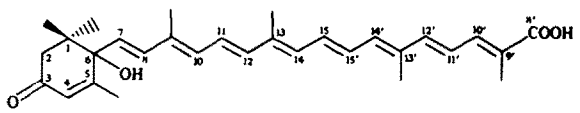
INTRODUCTION

Cochlospermum tinctorium A. Rich. (Syn. *C. niloticum* Oliv.) is one of the important Guinean medicinal plants used in the treatment and prevention of diseases due to liver damage [1, 2]. In a search for the active principle(s), large amounts of carotenoids were isolated from the rhizome. The presence of these pigments has been previously mentioned [3]; we now report the identification of the two major constituents, cochloxanthin (**1**) and dihydrocochloxanthin (**2**).

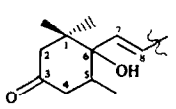
RESULTS AND DISCUSSION

Electronic spectra of **1** recorded in different solvents were in agreement with an octaenone chromophore as found in paracentrone [4]. Hypsochromic shifts of the VIS absorption maxima in alkali medium which were reversible by addition of acid [4] and IR absorption at 1660 cm^{-1} indicated the presence of a conjugated carboxylic function. MS data confirmed this hypothesis as the molecular ion of **1** at *m/z* 462 ($C_{30}H_{38}O_4$) was shifted to *m/z* 476 on methylation with diazomethane; moreover, the IR spectrum of the methyl ester showed absorption at 1695 cm^{-1} (conjugated ester) and the ^{1}H NMR spectrum exhibited an additional singlet at δ3.76 (3H).

The presence of a 3-keto-ε-carotene ring was suggested



1 Cochloxanthin



2 Dihydrocochloxanthin

by the ^{1}H NMR spectrum which showed a one proton broad singlet at δ5.93, two doublets at δ2.28 and 2.48 (J = 17 Hz) and a three proton doublet at δ1.91 (J = 1.3 Hz). This was further supported by the ^{1}H NMR data of all-*trans*-6,6′-dihydrorhodoxanthin [6, 7]; thus a hydroxy-substitution at C-6 of **1** was suggested by the downfield position of the doublet attributed to H-7 (δ5.74, J = 15.8 Hz) and the absence of the doublet at δ2.61 (H-6). The substitution by a hindered hydroxyl was further confirmed by the mass spectrum of a silylated derivative (*m/z* 548 [M]$^+$) which was obtained only with a strong silylating reagent (TSIM). Other ^{1}H NMR data provided evidence for four methyls in the side chain (δ1.92–2.00), two *gem*-methyls (δ1.03 and 1.11) and ten olefinic protons (δ6.20–6.78); the one proton doublet at δ7.40 was attributed to H-10′ in the β-position of a conjugated carbonyl as observed in 2′-dehydroplectaniaxanthin [8]. The ^{13}C NMR measurements (broad band and DEPT) supported the above data: signals between δ123 and 145 corresponding to 13 tertiary carbons, seven methyl signals between δ12.5 and 24.2, one secondary carbon signal at δ49.86 (C-2) and two carbonyl signals at δ198.05 (C-3) and 172.5 (C-8′) were observed.

Therefore, the structure 6-hydroxy-8′-apo-ε-caroten-3-one-8′-oic acid was attributed to **1** for which the name cochloxanthin was proposed.

In comparison with **1**, **2** showed a molecular ion at *m/z* 464 and similar spectroscopic data. However, the methyl at C-5 appeared as a doublet at δ0.96 (J = 6.11 Hz) in the ^{1}H NMR spectrum [9, 10] and the one proton broad singlet at δ5.93 was absent; in addition a two proton multiplet was observed between δ2.28 and 2.48 and a one proton multiplet appeared at δ1.23. Moreover, the ^{13}C NMR (DEPT) spectrum exhibited only 12 signals between δ123 and 145. These data confirmed the saturation of the double bond between C-4 and C-5 of **1**; thus the structure of 4,5-dihydro-6-hydroxy-8′-apo-ε-caroten-3-one-8′-oic acid was attributed to **2** for which the name dihydrocochloxanthin was proposed.

Although UV-VIS, IR and ^{1}H NMR data suggested an all-*trans* configuration for both compounds, ORD and

CD measurements are necessary to establish their absolute stereochemistry.

All the extracted carotenoids were in the free form as all attempts to saponify them were unsuccessful and each of them could be methylated with diazomethane. It was observed that the end groups of **1** were identical to those of abscisic acid. Furthermore the localization and the concentration (about 0.5%) determined by VIS spectrometry [11] of these unusual carotenoids in the rhizome is uncommon.

EXPERIMENTAL

Plant material. Rhizomes of *C. tinctorium* were collected in Guinea (Mamou, Middle-Guinea) in April 1984. The plant was identified at the 'Station Autonome de Sérédou' (Macenta, Guinea). A voucher specimen (number 1265) has been deposited at the herbarium of this centre.

Methods. All NMR spectra (^{1}H NMR at 250 MHz, ^{13}C NMR at 62.89 MHz) were obtained in $CDCl_3$ solns using TMS as the int. reference on a Bruker WP 250 instrument. MS spectra were recorded with a VG Micromass 7070 F apparatus (70 eV).

Methylation of **1** and **2** was achieved in Et_2O with CH_2N_2.

Silylation of **1** and **2** (1 mg) was performed with *N*-trimethylsilyl-imidazol (TSIM, 0.5 ml) in C_5H_5N (0.5 ml) at room temp. for 12 hr and the solns were evapd under a stream of N_2.

Isolation of carotenoids. Procedures and precautions for carotenoid manipulations were generally observed [12]. The air-dried rhizomes (200 g) were powdered and immediately extracted by percolation with MeOH. The extract was evapd under reduced pressure at a temp. below 30°. A TLC and an UV-VIS spectroscopic comparison of this extract with another one obtained from the fresh material showed no difference in the R_f values and the spectra of the main yellow pigments. The methanolic extract was defatted by successive washing with petrol then suspended in dried peroxide-free Et_2O. The Et_2O extract was evapd under reduced pressure. The major carotenoids of this extract were further separated by countercurrent chromatography (CCC) using an Ito-multilayer coil separator–extractor [13] equipped with a 2.6 mm i.d. column and a Shimadzu spectrophotometer UV-120, operating at 440 nm with a through flow cell, as detector; the solvent system was CCl_4–MeOH–H_2O (5:4:1) the lower phase corresponding to the stationary phase and the upper phase to the mobile phase (flow rate: 4 ml/min). **1** (14 mg) was recovered in a pure form; **2** (7 mg) and its Me ester were purified by HPLC (silica gel Si-60 5μ, mobile phase CH_2Cl_2–MeOH 99.5:0.5 at 1 ml/min, detection at 260 nm).

Purity of the fractions was checked by TLC either on silica gel 60 F_{254} (CH_2Cl_2–MeOH, 9:1) or on silica gel RP-8 (MeOH–H_2O, 9:1).

Estimation of carotenoids. The pigments present in the powder of the rhizomes (1 g) were exhaustively extracted with MeOH by percolation. The MeOH extract was concd to 10 ml and 1 ml of this soln diluted 1:100 with MeOH. The carotenoid content (% dried powder) was estimated from the *E* value of this dilution at 440 nm using 807 as the $E^{1\%}_{1cm}$ of **1** in MeOH at 440 nm.

6-Hydroxy-8'-apo-ε-caroten-3-one-8'-oic acid, cochloxanthin (**1**). VIS $\lambda^{CS_2}_{max}$ nm: (444), 470, 501; III/II(%) [14] = 46; VIS λ^{EtOH}_{max} nm: (414), 442, 468; III/II(%) = 20; VIS λ^{EtOH}_{max} nm (after alkalization): (410), 435, 463: III/II (%) = 72; IR ν^{KBr}_{max} cm^{-1}: 3420 (OH), 1660 (conjugated C=O), 1610 (–C=C–), 1395–1370 (*gem*-Me), 1260 (C=O), 965 (*trans* disubstituted double bonds); MS *m/z*: 462.279590 $[M]^+$ (calc. for $C_{30}H_{38}O_4$: 462.276991), 444.266430 $[M-H_2O]^+$ (calc. for $C_{30}H_{36}O_3$: 444.266428), 426 $[M-H_2O-H_2O]^+$, 418 $[M-44]^+$, 248.178570 (calc. for $C_{16}H_{24}O_2$: 248.177619); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.03 (*s*, 3H, Me-1), 1.11 (*s*, 3H, Me-1), 1.91 (*d*, $J_{Me\text{-}5,4}$ = 1.3 Hz, Me-5), 1.92–1.99 (9H, Me-9, Me-13, Me-13'), 2.00 (*s*, 3H, Me-9'), 2.24 (*d*, $J_{2,2}$ = 17 Hz, 1H, H-2), 2.48 (*d*, $J_{2,2}$ = 17 Hz, 1H, H-2), 5.74 (*d*, $J_{7,8}$ = 15.8 Hz, 1H, H-7), 5.93 (*s*, 1 H, H-4), 6.20–6.78 (*m*, 10 H, H-8, H-10, H-11, H-12, H-14, H-15, H-15', H-14', H-12', H-11'), 7.40 (*d*, $J_{10',11'}$ = 10.8 Hz, 1H, H-10'); ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 12.54, 12.73, 12.88, 13.17, 19.05, 23.05, 24.23 (Me-1/Me-1/Me-5/Me-9/Me-13/Me-13'/Me-9'), 49.89 (C-2), 123.27, 125.12, 126.88, 127.82, 130.00, 132.12, 133.03, 133.40, 135.53, 136.26, 138.77, 140.99, 144.92 (C-4/C-7/C-8/C-10/C-11/C-12/C-14/C-15/C-15'/C-14'/C-12'/C-11'/C-10'), 29.72, 41.67, 79.91, 134.24, 135.79, 137.66, 162.89 (C-1/C-5/C-6/C-9/C-13/C-13'/C-9'), 172.55 (C-8'), 198.06 (C-3).

4,5-Dihydro-6-hydroxy-8'-apo-ε-caroten-3-one-8'-oic acid, dihydrocochloxanthin (**2**). VIS λ^{EtOH}_{max} nm: (414), 440, 465; III/II (%) = 8; MS *m/z*: 464 $[M]^+$, 446 $[M-18]^+$, 420 $[M-44]^+$; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.90 (*d*, $J_{5,5}$ = 6.11 Hz, 3H, Me-5), 0.96 (*s*, 3H, Me-1), 0.97 (*s*, 3H, Me-1), 1.23 (*m*, 1H, H-5), 1.94–1.99 (12H, Me-9, Me-13, Me-13', Me-9'), 2.19–2.45 (*m*, 4H, H-2, H-4), 5.70 (*d*, $J_{7,8}$ = 15.9 Hz, 1H, H-7), 6.22–6.73 (*m*, 10H, H-8, H-10, H-11, H-12, H-14, H-15, H-15', H-14', H-12', H-11'), 7.40 (*d*, $J_{10',11'}$ = 10.61 Hz, 1H, H-10').

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