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Nuclear magnetic resonance analysis of carotenoids from the burgundy plumage of the Pompadour Cotinga (*Xipholena punicea*)

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ABSTRACT

Previous analysis of carotenoids extracted from the burgundy plumage of the Pompadour Cotinga (*Xipholena punicea*) revealed six novel keto-carotenoid pigments with methoxyl groups in the C3-position of one or both β-rings. High performance liquid chromatography (HPLC), mass spectrometry, chemical analysis and, in some instances ^{1}H NMR spectroscopy were employed to determine the structures of the molecules. Further analysis by NMR was precluded due to lack of material. The recent acquisition of multiple feathers from *X. punicea* specimens has made it possible to complete this work using correlated homonuclear spectroscopy (COSY), nuclear overhauser effect spectroscopy (NOESY) and ^{1}H NMR. These new data conclusively confirm the structures of the six methoxy-carotenoids suggested by the earlier work. In addition, the resonance positions of the protons from the novel 3-methoxy-4-keto-β-ring and 2,3-didehydro-3-methoxy-4-keto-β-ring moieties are reported here for the first time.

Introduction

The brightly colored plumages of birds contain a diversity of pigments, including a number of novel structures derived from four common dietary carotenoids, lutein, zeaxanthin, β-carotene and β-cryptoxanthin [1]. In a recent study [2], extraction and analysis of the pigments from the burgundy plumage of the male Pompadour Cotinga, *Xipholena* (*X.*) *punicea* revealed six previously unreported carotenoids with methoxyl groups in the C3 and C3′-positions. These pigments were proposed to be derived via three metabolic reactions, C4-oxygenation, C3(3′)-methylation and C2, C3-dehydrogenation. Whereas C4-oxygenation of dietary carotenoids is reported to be common in birds [1], the other two metabolic transformations are relatively uncommon for naturally-occurring carotenoids. Methoxylation of β-ring structures of carotenoids had previously only been reported in a few rare cases involving sponges [3–5] and more recently, in the human retina [6]. Carotenoids having a double-bond between C2 and C3, such as astacene (3,3′-dihydroxy-2,3,2′,3′-tetradehydro-β,β-carotene-4,4′-dione), phoeniconone (3-hydroxy-2,3-didehydro-β,β-carotene-4,4′-dione), and α-doradecin (3,3′-dihydroxy-2,3-didehydro-β,ε-caroten-4-one) are also relatively uncommon and are thought to be artifacts formed due to exposure to alkali or oxygen during sample processing [7]. However, a recent analysis of crimson and violet feathers from multiple species of Eurasian Broadbills has revealed a similar xanthophyll identified as 2,3-didehydro-papilioerythrinone [8], providing additional evidence that birds are in fact capable of carrying out the reaction that incorporates a double bond between C2 and C3.

Previous identifications of the carotenoids obtained from the solvent extracts of *X. punicea* plumage were accomplished using high performance liquid chromatography ([1]HPLC), mass spectrometry, chemical analysis and limited ^{1}H NMR spectroscopy [2] (The previous work is summarized in the Supplemental information of this article). Insufficient material was available at that time to carry out a detailed NMR structure analysis of the six novel carotenoids. The recent acquisition of multiple feathers from *X. punicea* specimens has made it possible to complete the NMR work.

In a typical ^{1}H NMR spectrum, the chemical shift, multiplet structure, spacings between the resonances, and integration of the peaks in a ^{1}H NMR spectrum can be used to determine the bond status and connectivity of protons in a molecule which can aid in the elucidation of its structure. The 2D-NMR spectroscopic methods of correlated homonuclear spectroscopy (COSY) and nuclear overhauser effect spectroscopy (NOESY) can be used to obtain even more detailed structural information by analyzing the coupling of protons associated with one or more bonds. COSY elucidates spin-couplings between protons that are connected

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[1] *Abbreviations used:* HPLC, high performance liquid chromatography; COSY, correlated homonuclear spectroscopy; NOESY, nuclear overhauser effect spectroscopy; MTBE, mixture of methyl tert-butyl ether; NP, normal phase; RP, reverse phase.

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through a single bond [9]. NOESY examines relationships between protons that are spatially coupled across multiple bonds [10,11]. The present study uses ^{1}H, COSY, and NOESY NMR to provide detailed structural identifications of the novel, methoxy-containing carotenoids from *X. punicea*.

Methods

Extraction of pigments

Two deceased male *X. punicea* specimens (YPM 141968 and 142924) were obtained by the Yale Peabody Museum from the Dallas World Aquarium and Zoological Garden where they had been living in captivity. The burgundy feathers of these specimens were plucked and soaked in 1 L of technical grade ethanol for 30 min, after which time they were transferred to filter paper and patted dry. The feathers obtained from specimen YPM 141968 were placed in 1 L fresh ethanol for an additional 30 min, because the feathers contained a significant amount of dirt and debris. Both sets of feathers were then soaked in 1 L of technical grade hexanes for 30 min and then patted dry in the same fashion. It should be noted that the feathers of specimen YPM 142924 released some pigment upon treatment with hexanes, which was evidenced by a slight orange coloration of the solvent and filter paper.

Subsequently, the pigmented barbs were trimmed and divided into 8 roughly equal lots. Each lot was placed into a 50 mL screw-cap glass jar, covered with ~30 mL of acidified pyridine and heated in a 90 °C water bath for 90 min, after which time

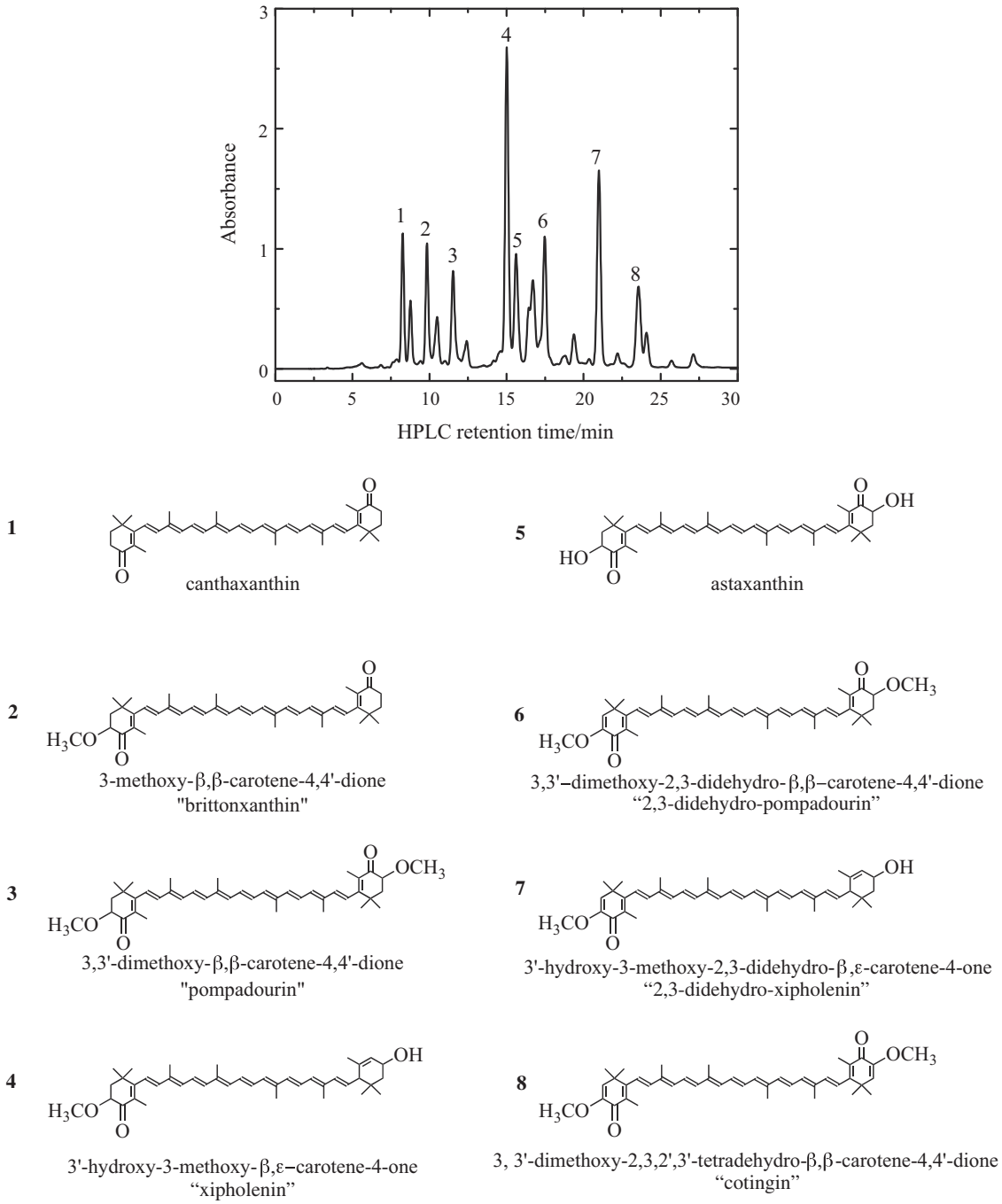

Fig. 1. Preparative NP-HPLC chromatogram of *X. punicea* extract, detected at 450 nm, with eight major peaks denoted numerically. Proposed structures are also given as reported in [2].