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Chirality of 2-(4-Methoxyphenoxy)propanoic Acid in Roasted Coffee Beans: Analysis of the Methyl Esters by Chiral High-Performance Liquid Chromatography

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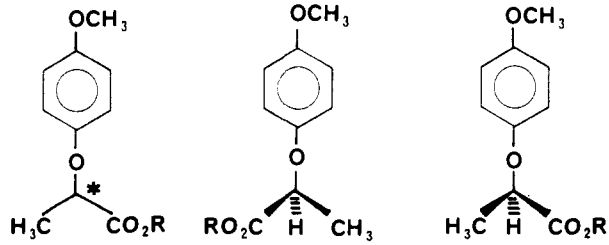
Methyl 2-(4-methoxyphenoxy)propanoate, previously shown to be present in methylated aqueous extracts of roasted Colombian Arabica coffee beans, was purified by reversed-phase high-performance liquid chromatography (HPLC) with a Chromspher C_{18} column (250 × 4.6 mm). The purified extract was analyzed by chiral HPLC with a Chiralcel OK column (250 × 4.6 mm), which provided excellent separation of the two enantiomers of the ester. The methyl 2-(4-methoxyphenoxy)propanoate in the methylated coffee extract was shown to be composed of predominantly (80%) the (*S*)-(−) enantiomer.

A new flavoring, 2-(4-methoxyphenoxy)propanoic acid, which is the subject of Tate & Lyle PLC's British Patent Application No. 2157148A and corresponding patent applications worldwide (Lindley and Rathbone, 1985), has recently been identified in aqueous extracts of roasted Colombian Arabica coffee beans (Rathbone et al., 1989). This flavoring (**1**) has an asymmetric carbon atom and can therefore exist in two enantiomeric forms: (*S*)-(−) and (*R*)-(+) (**3** and **5**, respectively). Sensory evaluation of the individual enantiomers (**3** and **5**) has shown that the important flavoring functionalities (Rathbone et al., 1989) reside solely in the (*S*)-(−) form, the (*R*)-(+) enantiomer being functionally inert. To establish which enantiomer of 2-(4-methoxyphenoxy)propanoic acid occurs in roasted coffee beans, the extracts generated by the previous investigation (Rathbone et al., 1989) were methylated, purified by HPLC, and analyzed by chiral HPLC for the presence of the individual enantiomers of methyl 2-(4-methoxyphenoxy)propanoate.



1, R = H **2**, R = CH_3 **3**, R = H **4**, R = CH_3 **5**, R = H **6**, R = CH_3

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MATERIALS AND METHODS

Materials. (*S*)-(−)-2-(4-Methoxyphenoxy)propanoic acid (**3**) was prepared by resolution of the racemic acid (**1**) via its brucine salt (Fredga and Avalaht, 1965): mp 65–66 °C; $[\alpha]^{20}_D$ −42.7° (*c* 0.95, absolute EtOH); lit. (Fredga and Avalaht, 1965) mp 65–66 °C; lit. (Fredga and Avalaht, 1965) $[\alpha]^{20}_D$ −43.1° (*c* 2.17, absolute EtOH).

Methyl (*S*)-(−)-2-(4-methoxyphenoxy)propanoate (**4**) was prepared by treatment of **3** (2.0 g) with methanol–HCl (1%, 6 mL) at 60 °C for 0.5 h. CH_2Cl_2 (50 mL) was added to the cooled solution and the mixture extracted with saturated aqueous $NaHCO_3$ solution (2 × 20 mL). The CH_2Cl_2 layer was washed with water (2 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo to afford the product (**4**) as a pale yellow liquid: 2.0 g; $[\alpha]^{20}_D$ −71.4°, $[\alpha]^{20}_{365}$ −282.8° (*c* 2.3, acetone); EI MS, *m/z* (relative intensity) 210 (M^+, 63), 151 (39), 124 (53), 123 (100), 109 (34), 95 (16), 77 (15); ^{1}H NMR δ 6.82 (4 H, H-2′, 3′, 5′, 6′, unresolved m), 4.67 (1 H, q, H-2, $J_{2,3}$ = 6.8 Hz), 3.752, 3.747, (2 × 3 H, 2 s, CO_2CH_3, 4′-OCH_3), 1.59 (3 H, d, H-3, $J_{3,2}$ = 6.8 Hz); ^{13}C NMR δ 172.7 (C-1), 154.4 (C-1′), 151.5 (C-4′), 116.3 (C-2′, 6′), 114.5 (C-3′, 5′), 73.4 (ester CH_3), 55.4 (4′-OCH_3), 52.1 (C-2), 18.5 (C-3).

Extracts A–C and (*R,S*)-(±)-2-(4-methoxyphenoxy)-propanoic acid (**1**) and its methyl ester (**2**) were obtained as previously described (Rathbone et al., 1989). HPLC-grade solvents were used throughout. Solvent-resistant filters were purchased from Schleicher and Schuell (West Germany).

HPLC Analysis. An LKB HPLC system was used, fitted with two 2150 pumps, an LC 2152 controller, and a rapid spectral detector (2140), scanning 190–370 nm. LKB wavescan (2140-250 version 1.08) and Nelson chromatography (2145-200) software was used to process the data on a Wang PC with IBM PC emulation option (version 3.2). Injection was via Rheodyne injector loops (20 or 250 μL). Either of the following columns was used at room temperature. Column A: Chrompak Chromspher C_{18} HPLC column (250 × 4.6 mm). Elution program: (a)

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initial composition of chloride buffer–methanol (70:30, v/v) changing linearly over 25 min to 45:55 (v/v), (b) buffer to methanol ratio (45:55) maintained for 10 min, (c) composition changed to 20:80 buffer/methanol (v/v) over 1 min, and (d) the latter composition maintained for 9 min to the end of the run (run time 45 min). The gradient was changed to achieve the initial composition (70:30, v/v) over 1 min, which was maintained for a further 9 min prior to the next injection (total cycle time 55 min). Chloride buffer composition: NaCl (5.74 g) dissolved in 0.002 M HCl and diluted with methanol (10% v/v in the buffer solution). Flow rate: 1 mL/min maintained throughout. Column B: Chiralcel OK column (Daicel Chemical Industries Ltd) (250 × 4.6 mm). Eluent: *n*-hexane–2-propanol (90:10, v/v), isocratic. Flow rate: 1 mL/min. The run time was 45 min.

GC–MS. GC–MS analysis was performed on a Kratos MS80RF/DS55 mass spectrometer and data system, directly coupled to a nonpolar bonded phase (BP5, 5% methyl phenylsiloxane) fused silica column (50 m × 0.33 mm (i.d.)) supplied by Scientific Glass Engineering Ltd. The GC was temperature-programmed 1 min at 100 °C, 16 °C/min to 250 °C, 10 min at 250 °C; helium at 0.36 kg/cm^2 was used as carrier gas. Injection volumes were 1 μL, with splitless injection at 200 °C. MS scan rate was 1 s/decade, interscan time 1 s, range m/z 30–800.

Direct-insertion mass spectra were recorded on the same instrument, operating at 70 eV with a source temperature of 200 °C and using a heated direct-insertion probe.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker WM 250 spectrometer, with $CDCl_3$ as solvent. Chemical shifts are referenced to the solvent at 77.0 and 7.24 ppm, respectively, for ^{13}C and ^{1}H, and are reported (ppm) relative to tetramethylsilane.

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in a 1-dm cell.

Preparation of Extracts. The preparation of extracts A–C from roasted Colombian Arabica coffee beans was described in a previous paper (Rathbone et al., 1989). The remainder of extract A (ca. 700 μL) was evaporated to dryness, methylated (500 μL of 1% methanol–HCl), and combined with extracts B and C. The combined, methylated extract (extract D) was filtered (0.45 μm) and purified by reversed-phase HPLC prior to analysis by chiral HPLC.

HPLC Purification. Extract D (total volume 1.0 mL) was chromatographed on column A in five injections (5 × ≈185 μL), and samples of the eluate were collected at 1-min intervals, spanning the elution time of methyl 2-(4-methoxyphenoxy)propanoate (**2**) (ca. 21.5 min). The appropriate fractions from the five runs were combined (total volume 5 mL) and extracted with *n*-hexane–diethyl ether (2:1) (4 × 0.5 mL). The combined organic extract was concentrated in a vacuum desiccator to a volume of 0.5 mL (extract E).

Preparation and Purification of Extracts. The extraction of roasted Colombian Arabica coffee beans with hot water and subsequent purification of the extract by solvent extraction and preparative-layer chromatography are described in a previous paper (Rathbone et al., 1989). This work yielded extracts A (an acetone solution containing the free acid **1**) and extracts B and C (methylated aliquots of extract A). The use of chiral HPLC to determine the chirality of the natural 2-(4-methoxyphenoxy)-propanoic acid was investigated. None of the chiral HPLC columns evaluated gave adequate separation of the enantiomers of the acid; however, Chiralcel OK was found to give excellent resolution of the enantiomers of the methyl ester (**2**). Chiral HPLC analysis was therefore performed on methylated samples of coffee extracts.

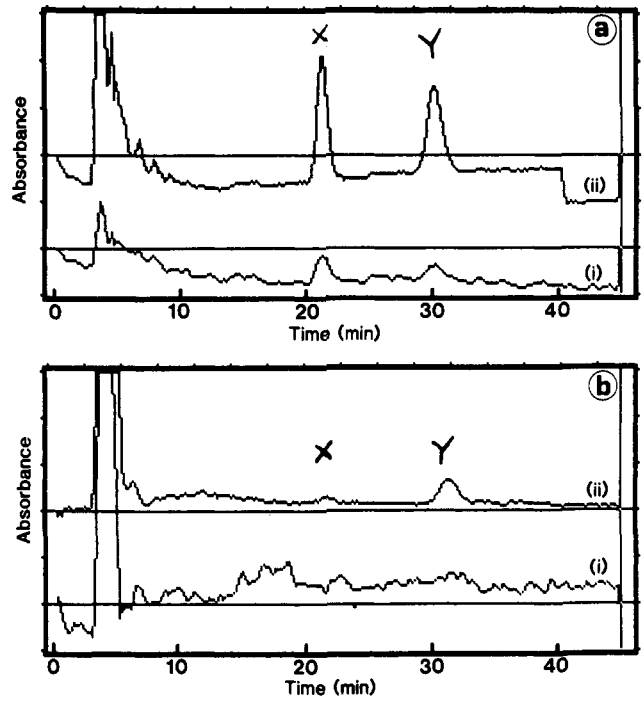

Figure 1. Chiral HPLC traces (column B): (a) 250 μL of a 2 ppm solution of methyl (*R,S*)-(±)-2-(4-methoxyphenoxy)propanoate (**2**) standard, peak at Y identified as the (*S*)-(−) enantiomer; (b) 250 μL of extract E, showing two peaks at X and Y corresponding to those of compound **2**. In both figures, the lower traces (i) were monitored at 225 nm whereas the upper traces (ii) are the background-subtracted chromatograms (see text).

Combination of extracts B and C with methylated extract A yielded extract D which was purified by reversed-phase HPLC (column A). The combined eluate was extracted with *n*-hexane–diethyl ether to separate the components of interest from the buffer. The concentrated extract (extract E) was analyzed by GC–MS (to confirm the presence of methyl 2-(4-methoxyphenoxy)propanoate in the purified extract) and by chiral HPLC.

RESULTS AND DISCUSSION

GC–MS Analysis. The GC trace of extract E, monitored at m/z 210, 123, and the total ion current, showed a peak at the same retention time as compound **2** (10.3 min). The fragmentation pattern of the mass spectrum corresponding to this peak was essentially the same as that of methyl 2-(4-methoxyphenoxy)propanoate, showing characteristic ions at m/z 210, 151, 123, and 109 and confirming the presence of ester **2** in extract E.

Chiral HPLC Analysis. The Chiralcel OK column produced excellent separation of the enantiomeric methyl esters **4** and **6**; the retention times were 30.3 and 21.3 min, respectively, which correspond to an enantiomeric separation factor (α) (Bishop et al., 1986) of 1.48 (Figure 1a).

The peaks were identified by injection of a solution of the (*S*)-(−) enantiomer (**4**), which produced a single peak at the retention time of the slower of the two enantiomers. This observation also demonstrates that acid **3** does not racemize under the methylation conditions employed.

Extract E (250 μL) was analyzed by chiral HPLC. Background absorption and noise variation were minimized by use of the LKB wavescan arithmetic subtraction routine [$F(t) = A(225\text{ nm}) - 0.9A(245\text{ nm})$] in which irrelevant absorptions (245 nm) were subtracted from those

of interest (225 nm). The resulting chromatogram (Figure 1b) showed the presence of both enantiomers **4** and **6** at the same retention times as those in the standard mixture but in unequal amounts, the (*S*)-(−) enantiomer being present at a higher concentration than the (*R*)-(+) ester. The two peaks were integrated with use of Nelson difference and integration programs, which quantified the peak area ratio for the (*S*)-(−) and (*R*)-(+) components as ca 4:1.

The natural form of 2-(4-methoxyphenoxy)propanoic acid, which occurs in Colombian roasted coffee, analyzed as the methyl ester, is therefore predominantly the (*S*)-(−) enantiomer.

Registry No. 4, 4276-74-8; **5**, 117896-90-9; (*R*)-(+)-2-(4-methoxyphenoxy)propanoic acid, 4276-75-9.

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Oat Phenolics: Avenanthramides, Novel Substituted *N*-Cinnamoylanthranilate Alkaloids from Oat Groats and Hulls[1]

F. William Collins

Fractionation of methanolic extracts of oat groats and hulls by anion-exchange chromatography revealed the presence of a series of anionic, substituted cinnamic acid conjugates, trivially named avenanthramides. Two-dimensional thin-layer chromatography (TLC) showed groat extracts contain more than 25 distinct avenanthramides, while hull extracts contained about 20. Some 15 were common to both groat and hull preparations. The substances were purified by repeated column chromatography on Sephadex LH-20, using TLC to monitor purity, and crystallized from aqueous acetone. The complete structures of 10 avenanthramides have been elucidated using ^1H and ^{13}C nuclear magnetic resonance (NMR), mass spectroscopy (MS), ultraviolet absorption spectroscopy (UV), and hydrolytic techniques and confirmed by total synthesis. The physicochemical properties, potential biological activity, and distribution within the oat grain are discussed.

Despite the widespread utilization of oats in the agri-food sector, little work has been done to characterize oat grain phenolic compounds. The structurally dependent involvement of cereal grain phenolics in a wide variety of biochemical and nutritional processes makes a knowledge of the types and amounts of these phenolics extremely valuable. Such information is important in improving the quality of specalized oat-based feed and food ingredients, in developing oat processing technology, in evaluating dietary input data for human and animal nutrition, and as an aid in elucidating mechanisms of disease resistance. An in-depth study of oat grain phenolics is therefore currently being carried out with particular emphasis on the structural elucidation of "bound" forms of the commonly reported hydroxycinnamic acids. The structures and functionality of some of these conjugated phenolics have recently been summarized (Collins, 1986), but a new group of nitrogen-containing constituents, the avenanthramides, have now been detected. The purpose of this paper is to briefly describe their occurrence, isolation, structure, physicochemical properties, and potential biological activity.

EXPERIMENTAL SECTION

Melting points were obtained on a Fisher-Johns apparatus and are uncorrected. Chromatographic separations of extracts and synthetic mixtures were carried out on Sephadex LH-20 (Pharmacia, Canada) using volume-calibrated glass columns and gravity-flow isocratic elution. Solvents used for column chromatography were as follows: I, $CHCl_3$–cyclohexane–MeOH–acetic acid (50:40:5:5); II, $CHCl_3$–cyclohexane–MeOH–acetic acid (50:35:10:5); III, $CHCl_3$–cyclohexane–MeOH–acetic acid (50:30:15:5); IV, $CHCl_3$–cyclohexane–MeOH–acetic acid (50:25:20:5); V, acetone–H_2O–acetic acid (30:65:5).

Chromatographic mobilities were recorded as relative elution values, K', equal to the ratio V_e/V_b, where V_e is the observed elution volume and V_b is the total volume of the packed gel (i.e., bed volume). When small sample application volumes V_c are maintained, relative to V_b (i.e., $V_c/V_b \leq 10\%$), the K' values were found to be independent of the size of the columns used and the process could be scaled up or down as required. Analytical TLC was performed on silica gel plates (Baker-Flex, 1B2-F, Canadian

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