TRITERPENES, WAXES AND TRICIN IN *PHOENIX CANARIENSIS*[1]

BEGOÑA GARCIA, J. ALBERTO MARCO, ELISEO SEOANE and AMPARO TORTAJADA

Department of Organic Chemistry, University of Valencia, Spain

ABSTRACT.—Investigation of the hexane and ethanol extract of the leaves of *P. canariensis* resulted in the isolation of two waxes, one insoluble and another soluble in ethanol, as well as aliphatic hydrocarbons, free long-chain acids, β-sitosterol, stigmasterol, lupeol, ferulic acid and the flavone tricin.

Phoenix canariensis Chabaud is an ornamental tree, very common in the Valencia country (Spain). Previous work on this plant included the chromatographic detection (but not isolation) of flavonoids (1, 2) and waxes (3) in a general taxonomic study of Palmae. The cutin (the high polymer which covers the leaves of the plant) has also been recently studied (4). In the present work, the leaves of *P. canariensis* were successively extracted with hexane and ethanol and their chemical components studied.

Components of the hexane extract. The hexane extract was separated by crystallization from hot ethanol (see experimental) into an insoluble wax and a mixture of ethanol-soluble components. The insoluble wax was shown by ir spectroscopy to contain only aliphatic esters (intense band at 1730 cm^{-1} and absence of any hydroxyl band). Presence of hydrocarbons was also ruled out by column chromatography on silica gel (see experimental). After saponification, the acids and alcohols were separated, transformed into their methyl esters and acetates, respectively, and analyzed by gc. Saturated straight-chain homologous series were found in both cases. The linear relationship between retention time and carbon number was graphically constructed for the saturated *n*-alkanoic acids with methyl esters of standard C_{16}, C_{18}, C_{22} and C_{24} acids, and for l-alkanols with the acetates of standard C_{24}, C_{28} and C_{30} 1-alkanols. By comparison of the retention times of the individual components of the mixture with those of the standard samples, the following results were found (only the main components are indicated): 1-alkanols (C_n % amount), C_{30} 7.9, C_{32} 38.9, C_{34} 31.7; n-alkanoic acids, C_{16} 12.8, C_{17} 4.5, C_{24} 24.4, C_{26} 28.0, C_{28} 15.4, C_{30} 4.3.

The ethanol-soluble part of the hexane extract was divided into neutral and acidic fractions. The acidic part was methylated and studied as described above by gc with reference samples: saturated *n*-alkanoic acids C_{15} (6.7%) and C_{16} (16.0%) as well as the unsaturated oleic (6.2%), linoleic (18.3%) and linolenic (41.0%) acids were found to predominate. The neutral part was chromatographed on a silica gel column, and four main fractions were obtained (see experimental). Fraction I was a mixture of homologous straight-chain saturated hydrocarbons. Gc analysis and comparison with standard samples (C_{24} and C_{29}) led to the following results (C_n% amount): C_{30} 25.4, C_{31} 31.1, C_{32} 16.0, and C_{33} 13.9 were the most abundant components. Fraction II (ir ν max 1735 cm^{-1}) was a wax soluble in ethanol. It was analyzed as described above for the insoluble wax, with the following results: n-alkanoic acids C_{16} (13.3%), C_{18} (33.1%), C_{20} (9.6%) and

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linoleic acid (37.3%) made up predominantly the acidic part of the esters, while n-triacontanol C_{30} was the main constituent (85.0%) of the alcoholic part.

Fraction III was a crystalline white substance, mp 132–136°, which gave a typical Liebermann-Burchardt test of sterols. The spectral behaviour was very similar to the known product β-sitosterol. Comparison by gc with a mixture of phytosterols of another origin (5) showed our product to be mainly a mixture of β-sitosterol and stigmasterol with a minor amount (~5%) of two other components, which were eventually identified as cholesterol and stigmastanol (5α-stigmastan-3-β-ol) by gc comparison with commercial samples (see experimental). Mixtures of β-sitosterol and stigmasterol, often accompanied with campesterol or cholesterol, were erroneously referred to in the literature as a single compound named "γ-sitosterol", as pointed out by Linde *et al* (6).

Fraction IV was a crystalline compound, mp 208–213°, $[\alpha]^{20}D+21°$, identified as lupeol by spectral and direct comparison with an authentic sample, kindly provided to us by Prof. A. G. González (7b).

Components of the ethanolic extract. The ethanolic extract was divided into neutral and acidic fractions. The former was neglected because of the small amount. The acidic part was studied by column chromatography on silica gel. Only two crystalline compounds were isolated. The first one, mp 169–170°, was shown to be ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid) by spectral and direct comparison with a synthetic sample. To the second product, mp 288–292°, the structure of the flavone tricin (5,7,4'-trihydroxy-3',5'-dimethoxyflavone) was assigned on the basis of its spectral properties (8, 9, 10).

EXPERIMENTAL[2]

PLANT MATERIAL.—Leaves from *P. canariensis* Chabaud were collected during June 1976 at Los Viveros, Valencia, Spain. The plant material was authenticated by Prof. J. Mansanet, Department of Botany, University of Valencia. Specimens can be found in the herbarium of Los Viveros.

EXTRACTION AND FRACTIONATION OF *P. canariensis* LEAVES.—The dried powdered leaves (1 kg) of *P. canariensis* were extracted exhaustively in a Soxhlet with hexane and then 95% ethanol. The hexane extract was evaporated *in vacuo*, and the residue (29 g) was dissolved in hot 95% ethanol (1 liter). Upon cooling, a greenish waxy product precipitated out. Repeated precipitations from ethanol gave a slightly colored wax (10.1 g) which was chromatographed on silica gel. Hexane eluted no product (this excluded the presence of hydrocarbons). Benzene eluted 10 g of a white product with the same melting interval and ir spectrum (ν max 2920, 2850, 1730 cm^{-1}) as the original wax. The wax was then submitted to analysis as described in the text. The filtrate from the precipitation of the wax was evaporated *in vacuo*, dissolved in ether, and exhaustively extracted with 10% aqueous NaOH. In this way, the acidic (4.6 g) and neutral compounds (11.9 g) were separated. The acidic part was methylated (CH_2N_2), dissolved in benzene, filtered through a small silica gel column and analyzed by gc as described in the text. The neutral part was chromatographed on a silica gel column, four main fractions have been obtained with the indicated eluents: I, 0.51 g (hexane); II, 1.7 g (hexane-benzene 1:1); III, 0.31 g (benzene-ether 99:1); and IV, 2.48 g (benzene-ether 9:1). No other crystalline

[2]Ir spectra were recorded in KBr pellets on a Perkin Elmer 281 spectrophotometer and uv spectra in MeOH solution on a Perkin Elmer 575 spectrophotometer. ^1H nmr spectra were recorded (60 MHz in $CDCl_3$ unless otherwise stated) with TMS as internal standard on a Perkin Elmer R-12B instrument. Mass spectra were taken with a Hitachi-Perkin Elmer RMU-6L spectrometer operating at 70 eV. Optical rotations were measured in $CHCl_3$ on a Perkin Elmer 141 polarimeter. Melting points were determined on a Koffler's hot stage apparatus and are not corrected. Column chromatography was performed on silica gel Merck (0.063–0.200 mm). Gc analyses were performed in the following conditions: a) wax compounds, two 2m, ⅛" columns, packed with 5% EGA and 3% OV-1 on Chromosorb W, column temperatures, 190° and 260° for the low and high molecular weight components, respectively; b) sterols, 2m, ⅛" column, packed with 3% OV-1 on Chromosorb W, column temperature, 270°; c) lupeol derivatives, the same column as in the sterols, column temperature, 280°, carrier gas, helium.

products were eluted with increased solvent polarity. Fractions I and II, identified as mixtures of hydrocarbons and esters, respectively, were analyzed as described in the text.

ISOLATION AND IDENTIFICATION OF THE STEROLS.—Fraction III was a white product, mp 132–136°, which gave a typical sterol response in the Liebermann-Burchardt test. Gc of the product showed four peaks with identical retention times to those of authentic β-sitosterol, stigmasterol (Fluka), cholesterol (Merck) and stigmastanol (5α-stigmastan-3β-ol, prepared by hydrogenation of stigmasterol). Gc combined with mass spectrometry of the individual peaks confirmed the structural attributions. Approximate percentages in the mixture were the following: β-sitosterol, 75%; stigmasterol, 20%; stigmastanol, 4%; and cholesterol, 1%.

ISOLATION OF LUPEOL.—Fraction IV was lupeol, mp 208–213°, (from MeOH), $[\alpha]^{20}D$ +21°; ir ν max: 3300, 1040 (sec OH), 3050, 1635, 880 cm^{-1} (C=CH_2); ^1H nmr (δ ppm): 0.6–2.4 (47H, br band, skeletal H), 3.2–3.4 (1H, m, C-3), 4.5–4.7 (2H, br d, C-30); ms, *m/e* (% rel. int.), M^+ *m/e* 426 for $C_{30}H_{50}O$ (19) and other ions at 411 (M-CH_3, 2), 408 (M-H_2O, 5), 218 (21), 207 (62), 189 (100). Identical (ir, tlc and mixture mp) with an authentic sample (7b). By the usual method (Ac_2O-pyr) an acetylated derivative was also prepared, mp 213–216°, ir ν max: 1735, 1250 cm^{-1}, identical (tlc, gc, ir and mixture mp) with and authentic sample (7b). Jones oxidation of the alcohol yielded a ketone, mp 164–168°, ir ν max: 1705 cm^{-1}, identical (ir, tlc, gc and mixture mp) with an authentic sample.

ISOLATION OF FERULIC ACID FROM THE ETHANOL EXTRACT.—The ethanol extract was evaporated *in vacuo*, dissolved in ether and extracted with 5% aqueous NaOH. A small amount (1.3 g) of neutral product was obtained but not studied. The acidic part (6.6 g) was chromatographed on silica gel and eluted with mixtures of hexane-ether. Only two crystalline products were obtained: hexane-ether 1:1 eluted 121 mg of ferulic acid, mp 169–170°, ir ν max 3370–2650, 1690 (COOH), 3400, 1200 (phenolic OH), 1620, 970 (conj. trans CH=CH), 1225, 1110, 1035 cm^{-1} (C-O-C); ^1H nmr at 80 MHz (δ ppm): 3.79 (3H, s, OMe), 6.30 (1H, d, *J*=15.7 Hz, Ar-CH=C*H*-COOH), 6.74 (1H, d, *J*=8 Hz, arom. H), 7.04 (1H, d, *J*=8 Hz, arom. H), 7.21 (1H, s, arom. H), 7.44 (1H, d, *J*=15.7 Hz, Ar-C*H*=CH-COOH); ms, *m/e* (rel. int.), M^+ *m/e* 194 for $C_{10}H_{10}O_4$ (100), 179 (M-CH_3, 30), 177 (M-OH, 14), 161 (M-CH_3-H_2O, 9), 133 (22); uv λ max (nm): 218, 234, 301sh, 320, shifted to longer wavelengths by adding base. Identical (mixed mp, ir and gc of the methyl ester) with a synthetic sample.

ISOLATION OF TRICIN.—Hexane-ether 1:3 eluted a yellowish crystalline product (34 mg), mp 288–292°, ir ν max 3610, 3300, 1175 (phenolic OH), 1652 (flavone C=O), 1260 (Ar-O-C), 840 cm^{-1} (Ar-H); ^1H nmr at 80 MHz in DMSO-d_6 (δ ppm): 3.86 (s, 6H, 2 OMe), 6.16 (1H, d, *J*=2 Hz, H-6), 6.51 (1H, d, *J*=2 Hz, H-8), 6.91 (1H, s, H-3), 7.27 (2H, s, H-2' and H-6'); ms, *m/e* (rel. int.), M^+ *m/e* 330 for $C_{17}H_{14}O_7$ (100) and other ions at 315 (M-CH_3, 5), 287 (M-CH_3-CO, 7), 178 (10), 152 (15); uv λ max (nm): 244, 271, 352, displaced by addition of NaOMe, $AlCl_3$, NaOAc/H_3BO_3 as expected for a 5,7,4'-trihydroxylated flavone (8).

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