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THE PHENOLIC ACIDS OF *PISTACHIA VERA**

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Key Word Index—*Pistachia vera*; Anacardiaceae; pistachio nut-shell; phenolic acids; anacardic acids; 6-*n*-tridecylsalicyclic acid; 6-[8(*Z*)-tridecenyl]salicylic acid.

Abstract—The solvent extract of the outer green shell of the pistachio nut, *Pistachia vera*, was found to contain a mixture of phenolic acids. From their ^1H and ^{13}C NMR spectra and accurate mass measurement, their structures were established as 6-alkyl and *cis* 6-alkenylsalicylic acids. The approximate composition was found by GC and HPLC to be: (13:0) 46%, (13:1) 17.4%, (15:0) 10.3%, (15:1) 7.3% and (17:1) 19.0%, anacardic acids. The double bond in the monoenoic acids was found by oxidative degradation to be at the 8-position. The predominantly C_{13} side-chain in the phenolic acids is novel.

INTRODUCTION

The pistachio nut, *Pistachia vera* is a member of the Anacardiaceae like the cashew nut, *Anacardium occidentale*. The shell composition of the latter has been extensively studied [2] and contains principally anacardic acid (**1**; $n = 0, 2, 4, 6$), a smaller proportion of cardol (**2**; $n = 0, 2, 4, 6$) and traces of C_{17} components. Until the present work this natural product appeared to be the only edible nut having a shell containing phenolic lipids. Although compositional studies have been reported on the fatty acids, carbohydrates and amino acids of pistachio kernels [3, 4], no studies on the shell composition have been described. Pistachio kernels of various Iranian types were found to have inappreciable compositional differences [5, 6].

Our investigations of the outer green shell from the pistachio nut have shown that it contains a substantial proportion of a novel C_{13} anacardic acid as the saturated (13:0) member (**3**; $n = 0$) and the *cis* monoenoic (13:1) member (**3**; $n = 2$), respectively 6-*n*-tridecylsalicyclic acid and 6-[8(*Z*)-tridecenyl]salicyclic acid. Small amounts of (15:0), (15:1) and (17:1) anacardic acids accompany the C_{13} constituents. C_{17} anacardic acids have been previously reported in *Pentaspadon motleyi* [7] and *P. officinalis* [8].

*"Long Chain Phenols", Part 24. For Part 23, see ref. [1].

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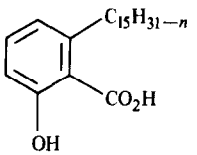
RESULTS AND DISCUSSION

Exhaustive extraction of finely ground shells from pistachio nuts yielded after removal of insoluble material about 10% of organic substances, a proportion lower than that in cashew shells (generally in the range 20–25%) [9]. TLC examination on silica gel revealed an upper spot with an R_f comparable to that of C_{15} anacardic acids from natural cashew nut-shell liquid and a smaller lower spot. Upon argentation TLC two pronounced upper spots were apparent (R_f 0.65, 0.55) approximately corresponding to (15:0) and (15:1) anacardic acids and two



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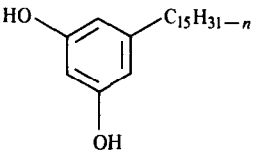

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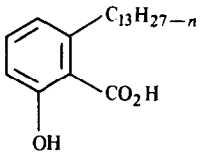

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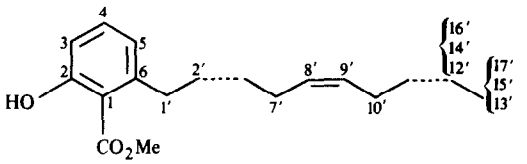

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lower fainter spots (R_f 0.35, 0.25). These were not removed after prolonged hydrogenation of the organic extract and their nature is uncertain, although they may well be a C_{13} analogue of cardol and a derivative of this substance.

The ^1H NMR spectrum of the main extract revealed a similarity to that of the C_{15} anacardic acids [10] although terminal vinylic and dienoid structural features were absent and only the monoene constituent was present in the *Z* configuration, from the *J* values exhibited. The splitting of the protons in the aromatic region closely resembled that found in the 1,2,3-trisubstitution of the natural C_{15} anacardic acids. The ^{13}C NMR spectral information was assigned on the basis of the structure (**4**), by off-resonance measurements, previous work [10] and the use of appropriate reference compounds. The infrared absorption spectrum of the methyl esters showed a hydrogen-bonded carbonyl band (1679 cm^{-1}) resembling that in (15:0) methyl anacardate (1678 cm^{-1}).

GC of the methyl esters of the pistachio acids and of the hydrogenated material on the stationary phase SE30 indicated C_{13}, C_{15} and C_{17} methyl anacardates by comparison of the retention times with those of synthetic methyl(6-*n*-tridecyl), methyl(6-*n*-pentadecyl), and methyl(6-*n*-heptadecyl)salicylate [11] respectively. With a semi-polar stationary phase (PEGA) some resolution of the saturated and monoenoic esters was obtained but this was better achieved with a wall-coated capillary column by GC/MS by means of which accurate mass determinations were made on the five peaks shown in Table 1.

In the fragmentation of the (13:1) methyl esters and other homologous constituents prominent ions were present at *m/z* 300, 166, 134 and 105, consistent with the general breakdown described [10] for anacardic acids.

HPLC separation by the reversed phase partition mode with methanol–water (10:1) on an octadecylsilane–silica gel column gave reasonable resolution of the methyl esters into the five major constituents having relative retentions similar to those found by GC. With the same column eluted with methanol–water–silver perchlorate the order of appearance of the (15:1) and (17:1) constituents was reversed and they emerged before the (13:0) and (15:0) constituents respectively. The relative retention and percentage composition (uncorrected) of the constituents by the two methods are shown in Table 2.

The separation of C_{15} anacardic acids in the polar non-hydrogen bonded structure has been observed early in the chromatogram when the reversed phase mode was used [12]. The addition of a small proportion of acetic acid to the solvent (e.g. methanol–water–acetic acid, 90:10:0.4) [13] enables C_{15} anacardic acids to be resolved by the reversed phase technique as a later group of less polar peaks since they then are present in the hydrogen-bonded form. However in more complex mixtures the level of acetic acid can be critical [V. Tychopoulos, J. H. P. Tyman and P. Chan, unpublished work]. The use of the methyl esters was advantageous to the present work since these materials are intramolecularly hydrogen-bonded [14] and suitable also for GC/MS analysis. They are appreciably more volatile than the methyl esters methyl ethers.

The double bond position in the unsaturated constituents could not be determined solely by ^1H NMR or ^{13}C NMR methods and an oxidative procedure was used on the derived methyl ester methyl ethers (**5**), consisting of dihydroxylation with performic acid, Malaprade oxidation of the vicinal diol followed by reduction of the aldehydes with sodium borohydride (Scheme 1). The reaction stages proceeded smoothly and were monitored either by TLC or ^1H NMR spectroscopy. Methyl 6-(8-hydroxyoctyl)salicylate methyl ether (**6**; $n = 7$) was obtained and identified by GC through comparison of its retention time with those of synthetic analogues [15] (**6**; $n = 2, 4, 6$). From the linear plot of log R_t versus methylenic chain length, the arylalkanol from the methylated pistachio acids was clearly an octanol derivative as seen from Table 3.

The accurate molecular mass determined by MS was in agreement with the required molecular formula, $C_{17}H_{26}O_4$. The aliphatic fragments consisting of a number of *n*-alkanols (**7**) arising from the different homologous chain lengths in the pistachio shell acids were identified by GC and MS as *n*-pentanol, *n*-heptanol and *n*-nonanol. The double bond in the various homologous monoenoic constituents is thus present at the 8-position as with the unsaturated constituents of *Anacardium occidentale* [2] and *Pentaspadon* species [7, 8].

Although C_{13} anacardic acids are novel, a C_{13} saturated resorcinol grevillol has been isolated from *Grevillea robusta* [16] and it is possible that traces of related materials accompany pistachio shell acids.

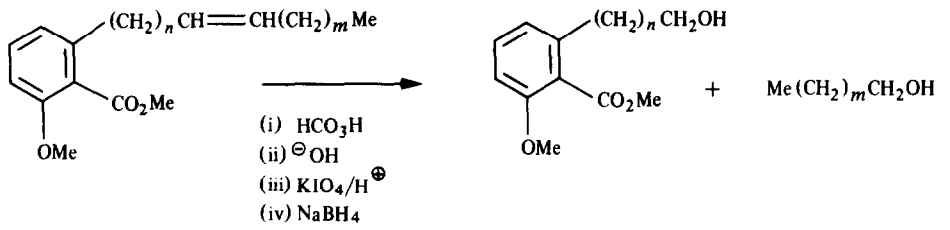
EXPERIMENTAL

Pistachio nuts were obtained from the Tehran area of Iran. TLC was carried out on silica gel with solvent A ($CHCl_3$–EtOAc, 19:1), solvent B (Et_2O–petrol (bp 40–60°)–HCO_2H, 30:70:2) and

Table 1. Capillary column GC and accurate mass determinations on methyl esters of anacardic acids from *Pistachia vera* shell

Peak*	Rel. retn.	$[M]^+$ Found	$[M]^+$ Reqd.	Formula	Side chain
4	9.20	332.2359	332.2359	$C_{21}H_{32}O_3$	$C_{13}H_{25}$
5	9.70	334.2522	334.2508	$C_{21}H_{34}O_3$	$C_{13}H_{27}$
6	11.12	360.2659	360.2662	$C_{23}H_{36}O_3$	$C_{15}H_{29}$
7	11.41	362.2834	362.2821	$C_{23}H_{38}O_3$	$C_{15}H_{31}$
8	12.89	388.2982	388.2977	$C_{25}H_{40}O_3$	$C_{17}H_{33}$

*Peak 2 (ref.) (Rel. retn. 1.0); peak 3 (trace).
Methylhexadecanoate (Rel. retn. 4.11; M^+ 270).



5 **6** **7**

Scheme 1.

Table 2. Relative retention (*RR*) and percentage composition (uncorrected) of the methyl esters of anacardic acids from *Pistachia vera* shell

Constituent	ODS column *RR*	ODS column %	ODS column with aq. MeOH $AgClO_4$ elution *RR*	ODS column with aq. MeOH $AgClO_4$ elution %
13:0	1.00	47.6	1.00	44.4
13:1	0.71	16.4	0.59	18.3
15:0	1.59	9.8	1.60	10.8
15:1	1.05	7.1	0.84	7.4
17:1	1.66	19.0	1.32	19.0

Table 3. Log R_t and methylenic chain length of synthetic standards and the arylalkanol from oxidative degradation of pistachio shell acids

Compound (6)	Log R_t
$n = 2$	1.38
$n = 5$	1.86
$n = 6$	2.02
Arylalkanol from *Pistachia* acids	2.19

solvent C (CH_2Cl_2–MeOH, 49:1) as indicated. Prep. TLC was effected on silica gel (1 mm layer) with 100 mg/plate. Bands were visualized with 0.1% ethanolic rhodamine 6G and eluted with Et_2O. Argentation TLC was carried out with $AgNO_3$–silica gel (1.5:10) with solvent B and bands were visualized with 50% aq. H_2SO_4 at 100°.

GC was conducted with a dual FID apparatus in which both the injector and the detector were 50° above the column temperature. N_2 was used at 40 ml/min with column A (3% SE30), column B (5% SE30) or column C (4% PEGA). Percentage composition by GLC was determined by triangulation of peaks. For GC/MS a WCOT capillary column (10 m) with OV101 was programmed from 60–260° at 4°/min; the mass spectra (EIMS) and accurate mass measurements were carried out on a Varian MAT 311 at 70 eV. The D22 service (PCMU, Harwell, England) was also used for certain accurate mass determinations. ^1H NMR at 100 MHz and ^{13}C NMR spectra at 25.2 MHz were determined on a Varian HA 100 instrument with TMS as int. standard.

IR spectra were determined as films. Column chromatography was effected on silica gel (350 mesh). HPLC was performed on an LC 711 Kipp and Zonen Chromatograph in the reversed phase partition mode on a 150:4.4 mm column, nucleosil-5-C_{18} (column A), at 95 bar with MeOH–H_2O (10:1) as solvent at 0.9 ml/min. Argentation HPLC was carried out on the same column with a 2.1 M $AgClO_4$, MeOH–H_2O soln (10:1). Detection in all cases was by UV absorption at 254 mm. Percentage composition by HPLC was obtained by triangulation of peaks.

Extraction. Finely ground dry shells (20 g) of pistachio nuts were exhaustively extracted with EtOAc in a Soxhlet apparatus. Evaporation of the solvent gave a brown syrup (2.1 g) which was suspended in CH_2Cl_2 (10 ml) and centrifuged to separate a yellowish solid (0.1 g). The soln was chromatographed on silica gel (100 g) with CH_2Cl_2 and with TLC monitoring. One main band (1.40 g) was recovered (R_f 0.3, solvent C). Further elution with CH_2Cl_2–MeOH (19:1) gave a brown fraction (0.3 g) the ^1H NMR spectrum of which mainly indicated aliphatic material.

TLC on the main material in solvent B showed a strong upper spot (R_f 0.80) and a fainter low spot (R_f 0.25). By argentation TLC (solvent B) two pronounced spots were observed (R_f 0.65, 0.55) similar to those of (15:0) and (15:1) anacardic acid [17] and two fainter spots (R_f 0.35–0.25). These latter were not removed upon hydrogenation of the pistachio acids (0.1133 g) in EtOH soln (15 ml) with Pd–C (10% Pd) (0.2252 g). The acids in ethereal solution at 0° with ethereal CH_2N_2 gave methyl esters which exhibited in EtOH soln a purple $FeCl_3$ soln reaction similar to that shown by (15:0) methyl anacardate and (15:0) methyl *iso*-anacardate.

GC with the hydrogenated pistachio acids esterified with CH_2N_2 at 0° gave on column A (230°) a major peak (C_{13}), retention 4.56 min, ref. (13:0) methyl anacardate 4.51 min; (15:0) 6.82 min; (11:0) 2.38 min. With column B (250°) the methyl esters of the hydrogenated acids showed C_{13}, 7.22 min; C_{15}, 11.2 min; C_{17} 18.6 min; ref. (13:0), 7.04 min; (15:0), 11.2 min and (11:0), 4.36 min. With column C (200°), peaks were C_{13}, 17.2 min, C_{15}, 31.5 min and C_{17}, 56.9 min; ref. (13:0), 16.9; (15:0), 30.0 min; (15:0) methyl *iso*-anacardate (methyl 4-pentadecylsalicylate) 35.2 min. The methyl esters of pistachio acids (before hydrogenation) showed peaks (column C, 200°) for (13:0) and (13:1), but the C_{15} peaks were not well resolved although in agreement with those in methyl anacardate, (15:0), 30.1 min; (15:1), 32.5 min; (15:2), 40.7 min and (15:3), 50.5 min.

The methyl ester methyl ethers of hydrogenated pistachio acids by phase transfer methylation (described in double bond determination) on column C (200°) gave C_{13}, 25.8 min; C_{15}, 46.2 min and C_{17}, 91.6 min. Percentage composition was obtained either by GC runs on SE30 or of hydrogenated methyl esters on PEGA. By GC on the hydrogenated methyl esters the composition (uncorr.) was found to be C_{13} (59.8%), C_{15} (19.4%) and C_{17} (20.8%), showing reasonable agreement with the HPLC results.

The phenolic methyl esters were analysed by HPLC and showed five major components with R_ts of 10, 15.2, 16.0, 24.2 and 25.6 min. Upon argentation HPLC the retention of

peaks 1, 3 and 4 were reduced to 9.2, 12.8 and 20 min respectively, as given in Table 2. GC/MS analysis of the same mixture gave the same five components with the retentions and mass spectral results shown in Table 1.

Spectroscopic properties. The IR spectrum (film) of the methyl esters showed ν (film) 1679 cm^{-1} (HO.....CO_2Me) compared with (15:0) methyl anacardate (1678 cm^{-1}) and (15:0) methyl *iso*-anacardate (methyl-4-pentadecylsalicyclate) (1689 cm^{-1}). The main acidic material had the following absorption. ^1H NMR (100 MHz, $CDCl_3$), δ0.87 (3H, *t*, Me), 1.25 [2OH, *m*, $(CH_2)_{10}$], 1.85–2.15 (0.8H, *m*, CH_2–C=), 2.85–3.05 (2H, *t*, CH_2Ar), 5.25–5.45 (0.4H, *m*, CH=CH, *cis*), 6.70–7.40 (3H, *m*, HAr) and 11.0 (1H, *br*, HO_2C, exch. D_2O)*.

The phenolic methyl ester (**4**) had a similar ^1H NMR spectrum with an additional band at δ3.9 (3H, *s*, MeO), and the following ^{13}C NMR (25.2 MHz, $CDCl_3$) with δ14.13 (*q*, C-13′) 22.77 (*t*, C-12′) 27.32 (*t*, C-10′, C-7′), 29.46, 29.65, 29.80, 29.99, 32.03 (C-11′, C-6′ to C-3′), 32.2 (*t*, C-2′), 36.7 (*t*, C-1′), 51.91 (*q*, MeO), 111.90 (3, C-1), 115.68 (*d*, C-3) 122.33 (dc, C-5), 129.79 (*d*, C-8′, C-9′), 134.10 (*d*, C-4), 146.13 (*s*, C-6), 162.83 (*s*, C-2) 171.92 (*s*, CO).

Determination of double bond position. Methylation of the chromatographed acids was effected more smoothly by the phase transfer method [10] than by the 'anhydrous' method. To the chromatographed acids (0.4355 g) in CH_2Cl_2 (10 ml) 3 M aq. NaOH solution (1.8 ml), 40% aq. tetra *n*-butylammonium hydroxide (0.55 ml) and Me_2SO_4 (1.2 ml) were added and the mixture vibromixed. After 2 hr the cloudy solution was substantially clearer. 3 M aq. NaOH (0.9 ml), 40% tetra *n*-butylammonium hydroxide (0.025 ml) and Me_2SO_4 (0.5 ml) were added and vibromixing continued for 1 hr (TLC showed nearly complete formation of the methyl ester methyl ether). The mixture was washed thoroughly with H_2O from ethereal solution and the dried ethereal extract concd. The residual oil was prep. TLC purified to remove Me_2SO_4 and for this, $CHCl_3$–petrol (40–60°) (1:3), was suitable. The recovered product (0.1233 g) possessed ^1H NMR absorption (CCl_4) similar to that of the original acid but with δ3.77, 3.80 (6H, 2*s*, OMe CO_2Me) in relation to δ6.57–7.33 (3H, *m*, HAr) and δ5.40–5.67 (1.5 H, *t*, CH=CH, J = 5 Hz) indicating the presence of the methyl ester methyl ether of the original acids.

The methyl ether methyl ester (0.077 g) in 99% HCO_2H (2 ml) at 0–10° was stirred during the slow addition of 30% H_2O_2 soln. After 3 hr (^1H NMR monitoring) the reaction was complete and the product recovered from the diluted soln by ethereal extraction was hydrolysed (to remove monoformate ester) by warming with 3 M aq. NaOH (2 ml) and H_2O (1.5 ml). The product from the diluted alkaline mixture was recovered by ethereal extraction and drying ($MgSO_4 \cdot H_2O$) to give (0.1006 g), ^1H NMR (CCl_4), δ0.77–1.0 (3H, *t*, Me), 1.33 [*m*, $(CH_2)_n$], 2.40–2.70 (2H, *t*, CH_2Ar), 3.17–3.40 [*m*, CH(OH)], 3.77, 3.80 (6H, 2*s*, OMe), 6.52–7.40 (3H, *m*, HAr), 2.20, 2.40 (*br s*, OH, exch. D_2O). Absorption due to CH_2CH= was absent.

The dihydroxy derivative of the methyl ester methyl ethers (0.0595 g), in MeOH (4 ml) was stirred and treated with 2% aq. HIO_4 (5 ml), from KIO_4 dissolved in 2 M aq. H_2SO_4. An almost immediate odour of a fatty alkanal was detectable. The mixture was kept overnight, diluted with water (5 ml) and ethereally extracted. The dried ethereal solution upon recovery gave a crude aldehyde mixture (0.0191 g) (R_f 0.9, solvent A). The crude aldehydes possessed ^1H NMR (CCl_4): δ8.03 (*s*, CHO) and were reduced in MeOH (3 ml) by stirring with $NaBH_4$ (0.0936 g). After 2 hr (TLC monitoring) reaction was complete and the mixture was diluted with H_2O, acidified and ethereally extracted. The dried ethereal solution was concd and prep. TLC purified. To locate the expected products, certain reference compounds were run alongside. In solvent A, five bands were present. Band 1 and band 2 contained traces of polar and polymeric material. Band 3 (R_f 0.30) had similar R_f to the reference compounds, methyl 6-(3-hydroxypropyl) salicylate methyl ether, methyl 6-(6-hydroxy hexyl)salicylate methyl ether and methyl 6-(7-hydroxy-heptyl)salicylate methyl ether. Under the partition conditions of GC, band 3 gave a single peak and from its R_t (31.2 min) in comparison with **6** (n = 2, 5, 6), respectively 4.81, 14.56 and 20.82 min (on column A, 180°) was methyl 6-(hydroxy-octyl)salicylate methyl ether (Table 3). EIMS (probe) 70 eV, m/z 294.1836 $[M]^+$, $C_{17}H_{26}O_4$ requires 294.1830. Band 4 was composed of a number of *n*-alkanols (**7**). GC/MS on 3% OV1 at 100° with He at 30 ml/min indicated the presence of *n*-nonanol. GC (column B) indicated small peaks ascribable to *n*-pentanol, *n*-heptanol and *n*-nonanol. Band 5 appeared to be composed of traces of the methyl ester methyl ether of (13:0), (15:0) and (17:0) anacardic acids.

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*The splitting of the aromatic protons (1,2,3-trisubstitution) was different from that in *iso*-anacardic acid (4-pentadecylsalycylic acid), a non-naturally occuring substance [A. A. Durrani and J. H. P. Tyman, unpublished work].