ALKYL AND PHENYLALKYL ANACARDIC ACIDS FROM *KNEMA ELEGANS* SEED OIL

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ABSTRACT.—In addition to triglycerides, the seed oil of *Knema elegans* Warb. (Myristicaceae) contains two series of anacardic acids. One series includes C_{11}, C_{13} and C_{15} saturated alkyl side chains and C_{15} and C_{17} monoenoid side chains. The other series is a previously unknown class of anacardic acids with a terminal phenyl group on a C_{10} or C_{12} saturated, or a C_{12} monoenoid connecting chain. Alkyl resorcinols, also present in the oil, appear to have the same side chains as the anacardic acids. The fatty acid composition of the oil is typical for a Myristicaceous plant in that myristic acid was dominant. Three lignans were also identified.

The Myristicaceae is a family of tropical evergreen trees from which one genus, *Myristica*, furnishes the nutmeg and mace of commerce (1). Uphof (2) describes five species of *Knema* as trees of Southeast Asia that are harvested for lumber and, although he does not include *K. elegans*, he mentions that seeds of one species (*K. corticosa*) are used to prepare medicinal salves. Seed oils from the Myristicaceae are noted for their high content of myristic acid (3). Our investigation of *K. elegans* oil was prompted when preliminary tests indicated the presence of large amounts of nonglyceride constituents. These components are now identified as anacardic acids and resorcinols, and include new classes of these compounds that have a terminal phenyl group on the side chains. Galbacin (4) and two related lignans were also identified.

RESULTS AND DISCUSSION

ANACARDIC ACIDS.—The known natural occurrence of anacardic acids (General structure **I**) has been limited to members of the Anacardiaceae and Gingkoaceae (5, 6). We now report a new source of anacardic acids that includes a new class of these compounds—those with phenylalkyl side chains. During chromatography on silica of *K. elegans* oil, anacardic acids were eluted immediately after the triglycerides (elution volume 150 to 350 ml). Isolation of the individual compounds was facilitated by conversion to acetylated methyl esters, which decreased their polarity and thereby made possible their separation by high-performance liquid chromatography (HPLC) on a reverse-phase column. For comparison, CNM the 15:1[8] anacardic acid (5) from cashew nut shell oil (CNM = cashew nut monoene) was isolated in exactly the same way.

Mass spectra of the anacardic acid derivatives are given in table 1. For analytical purposes, the trimethylsilyl (TMS) derivatives (silylated silyl esters) provide a convenient means to protect these acids from their propensity to decarboxylate (7) during gc analysis. The mass spectra of the TMS derivatives are characterized by ions for M–15 (M-CH_3), M–90 (M–TMSOH), and M–105 (M–CH_3 and TMSOH). Other features common to these spectra include ions at *m/e* 219 and 205 and, when the side chain is a phenylalkyl group, 91. The ions at *m/e* 205 and 219 probably result from side chain losses due to cleavages α and β to the anacardic ring together with loss of TMSOH. The ion at *m/e* 91 is the

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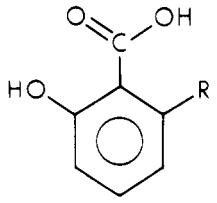


1

a. R = $-(CH_2)_{10}-CH_3$

b. R = $-(CH_2)_{12}-CH_3$

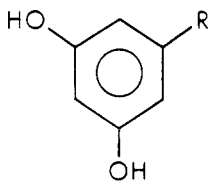
c. R = $-(CH_2)_{14}-CH_3$

d. R = $-(CH_2)_7-CH=CH-(CH_2)_5-CH_3$

e. R = $-(CH_2)_9-CH=CH-(CH_2)_3-CH_3$

f. R = $-(CH_2)_9-CH=CH-(CH_2)_5-CH_3$

g. R = $-(CH_2)_{11}-CH=CH-(CH_2)_3-CH_3$



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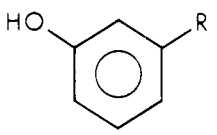
h. R = $-(CH_2)_{10}$ 

i. R = $-(CH_2)_{12}$ 



3

j. R = $-(CH_2)_7-CH=CH-(CH_2)_3$ 

CNM. R = $-(CH_2)_7-CH=CH-(CH_2)_5-CH_3$

TABLE 1. Major ions and relative intensities in the mass spectra of anacardic acid derivatives.

Compound	Derivative*	Mol. wt	Intensity				Other: Mass (intensity)
			M^-	$M-15^-$	$M-90^-$	$M-105^-$	
1a	TMS	436	4	100	5	7	265 (3) 219 (27), 205 (5), 180 (11)
1b	TMS	464	4	100	6	6	265 (5), 219 (36), 205 (6)
1c	TMS	492	3	63	3	3	293 (2), 265 (2), 219 (29), 205 (8), 73 (100)
CNM	TMS	490	15	100	20	6	293 (7), 262 (8), 233 (20), 219 (43), 205 (12)
1d, 1e	TMS	490	21	100	25	7	293 (5), 262 (5), 233 (8), 219 (39), 205 (8)
1h	TMS	498	9	100	11	6	293 (3), 265 (5), 219 (30), 205 (5), 91 (25)
1i	TMS	526	11	100	11	5	293 (4), 265 (4), 219 (29), 205 (4), 91 (28)
1f	TMS	524	27	100	37	7	343 (20), 293 (5), 281 (15), 265 (8), 219 (43), 208 (16), 91 (44)

TABLE 1. *Continued.*

Compound	Derivative*	Mol. wt	Intensity				Other: Mass (intensity)
			M^+	$M-32^+$	$M-42^-$	$M-74^+$	
1a	Ester	348	2	21	100	32	256 (8), 245 (7), 227 (6), 213 (4), 199 (7), 175 (13), 166 (27), 147 (34), 134 (22)
1b	Ester	376	2	21	100	23	284 (7), 273 (10), 259 (7), 175 (10), 166 (32), 161 (29), 147 (35), 134 (21)
1c	Ester	404	2	20	100	24	312 (8), 301 (8), 259 (4), 175 (9), 165 (31), 161 (27), 147 (34), 134 (20)
CNM	Ester	402	6	34	11	22	310 (24), 285 (2), 211 (8), 175 (7), 166 (45), 161 (21), 147 (33), 134 (23), 39 (100)
1d, 1e	Ester	402	6	37	11	22	310 (23), 285 (3), 211 (7), 175 (7), 166 (41), 161 (20), 147 (34), 134 (25), 39 (100)
1f, 1g	Ester	430	7	40	10	25	299 (6), 285 (3), 211 (5), 175 (7), 165 (19), 161 (26), 147 (34), 134 (22), 39 (100)
1h	Ester	410	5	24	27	19	318 (5), 227 (5), 188 (5), 175 (6), 166 (26), 161 (20), 147 (30), 134 (22), 91 (64), 39 (100)
1i	Ester	438	9	52	58	40	346 (23), 273 (6), 189 (6), 166 (39), 161 (27), 147 (48), 134 (28), 91 (100)
1j	Ester	436	7	15	7	18	300 (8), 271 (4), 221 (6), 219 (6), 208 (8), 197 (4), 187 (4), 173 (8), 166 (22), 161 (12), 147 (26), 134 (23), 91 (100)

*TMS = silylated silyl ester. Ester = acetylated methyl ester.

familiar tropylium ion (8). The acetylated methyl esters have mass spectral features of M–32 (M–CH_3OH), M–42 (M–CH_2CO) and M–74 (M–CH_3OH and CH_2CO). The prominent ion at *m/e* 166 probably has the same empirical formula as that noted in the spectra of acetylated alkylresorcinols (9). The ions at *m/e* 161 and 147 appear analogous to those at *m/e* 219 and 205 in the spectra of the TMS derivatives. Again, the tropylium ion is intense in spectra of phenylalkyl compounds.

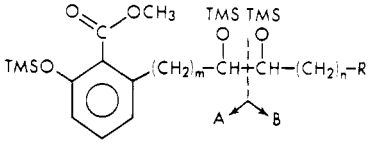
Infrared spectra of the anacardic acid mixture and of the acetylated methyl esters were identical to those of the anacardic acids and esters from cashew nut shell lipids. No evidence of *trans* unsaturation (950–1000 cm^{-1}) was found. Features of the proton magnetic resonance (pmr) spectra are given in table 2. Spectra of the anacardic acids **1d** and **1e** from *K. elegans* oil and CNM are virtually identical. **1b** has the resonances expected for an anacardic acid with a saturated side chain. **1j** has no terminal methyl triplet but, instead, has an intense multiplet at 7.15 ppm due to the protons of the phenyl ring. **1i** is clearly a saturated analog of **1j**.

TABLE 2. Proton magnetic resonances of acetylated methyl esters of anacardic acids in ppm.

Parent compound	CH_3	CH_2-C*H*-CH_2	C*H*$_2$—CH-Ar	C*H*$_2$-CH=	O ‖ C-CH_3	CH_2-Ar	O ‖ C-OCH_3	CH=CH	Aromatic
1b	0.87 *t*	1.25 *m*	1.55 *m*		2.20 *s*	2.72 *t*	3.82 *s*		6.83, 6.90, 6.99, 7.09, 7.25, 7.33
1d, 1e	0.88 *t*	1.28 *m*	1.57 *m*	1.95 *m*	2.20 *s*	2.72 *t*	3.82 *s*	5.28 *m*	6.83, 6.92, 6.99, 7.10, 7.26, 7.33
1i		1.26 *m*	1.55 *m*		2.15 *s*	2.70 *m*	3.82 *s*		6.84, 6.92, 6.99, 7.07, 7.14, 7.27, 7.33
1j		1.30 *m*	1.58 *m*	2.00 *m*	2.15 *s*	2.68 *m*	3.82 *s*	5.40 *m*	6.82, 6.88, 6.99, 7.10, 7.15, 7.28, 7.40
CNM	0.88 *t*	1.30 *m*	1.58 *m*	2.00 *m*	2.20 *s*	2.66 *m*	3.38 *s*	5.28 *m*	6.83, 6.90, 6.99, 7.08, 7.27, 7.34

All of the above data, taken together, show that **1b**, **1d** (**1e**), **1i** and **1j** are anacardic acids and that **1i** and **1j** have a phenylalkyl side chain. Structures **1a**, **1c**, **1f**, **1g** and **1h** were assigned on the basis of their mass spectra. Completion of the characterization needed only the location of the double bonds, which was accomplished by OsO_4 oxidation of the olefins followed by silylation of the resultant diols. OsO_4 oxidation and subsequent work up hydrolyzed the acetate ester in such a way that the final products were silylated methyl esters. Mass spectra of these compounds are given in table 3. Molecular ions were not ob-

TABLE 3. Pertinent ions in the mass spectra of silylated diols from anacardic acids.



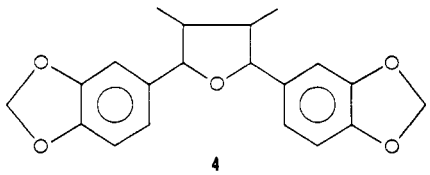
ReAr = A+73 (see ref. 10)

Parent compound	Mol. wt	Mass (intensity) M–15$^+$	M–31$^+$	ReAr$^+$	ReAr$^-$ –106	A$^+$	B$^+$	A– 104$^+$	Double bond position	R
CNM	610	595 (3)	579 (1)	496 (8)	390 (19)	423 (39)	187 (46)	319 (15)	8	CH_3
1d	610	595 (9)	579 (2)	496 (10)	390 (43)	423 (42)	187 (67)	319 (40)	8	CH_3
1e	610	595 (8)	579 (2)	524 (22)	418 (24)	451 (76)	159 (78)	347 (41)	10	CH_3
1f	638	623 (2)	607 (1)	524 (4)	418 (3)	451 (19)	187 (29)	347 (14)	10	CH_3
1g	638	623 (2)	607 (1)	552 (2)	446 (2)	479 (13)	159 (22)	375 (12)	12	CH_3
1j	644	629 (3)	613 (1)	496 (7)	390 (14)	423 (35)	221 (13)	319 (15)	8	Phenyl

served, but ions at M–15$^+$ and M–31$^+$ were evident. Cleavage between the diols locates the parent olefinic bonds. A prominent ion in all of the spectra must result from a rearrangement of a trimethylsilyl group to the carboxyl function (ReAr) in a manner similar to that observed in the spectra of fatty acid esters (10). An ion representing loss of 104 AMU from cleavage ion A (TMSO+CH_3) was intense in these spectra. These derivatives of **1d** and **1e** were nearly separated by gc and appeared in the approximate ratio of 95:5 (**1d**:**1e**). Likewise, **1f** and **1g** were estimated to be nearly equal in relative proportion.

RESORCINOLS AND CARDANOLS.—Five components (**2b**, **2d** (**e**), **2h**, **2i** and **2j**) were assigned resorcinol structures on the basis of the mass spectra of their TMS derivatives. The base peak at *m/e* 268 has been shown to be indicative of resorcinol substitution (9). Other features of the spectra are consistent with these structural assignments. Their occurrence in *K. elegans* is not surprising because the related resorcinols are found in other oils containing anacardic acids (6). Likewise, cardanols (structure **3**) with similar side chains were indicated by mass spectra. Since anacardic acids are easily decarboxylated to cardanols (7), these compounds may be artifacts produced during treatment of the samples.

LIGNANS.—A number of lignans were found in the petroleum ether extract. Optical rotation, melting point, PMR and mass spectrum of **4** clearly demonstrate that this compound is galbacin (4, 11). Compounds **5** and **6** were not crystallized,



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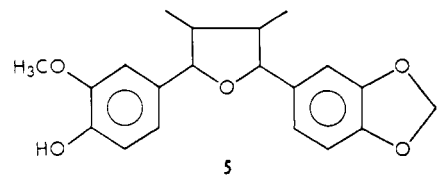

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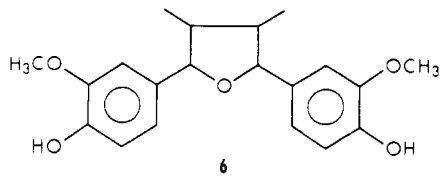

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but their pmr and mass spectra (TMS derivatives) are reasonable for these structures. Stereochemistry about the furanoid ring is probably the same as that of galbacin, and other workers have assigned the 3-methoxy, 4-hydroxy substitution pattern based on biogenetic grounds (12). The intense ion at *m/e* 264 is analogous to the rearrangement leading to *m/e* 190 in galbacin (11).

FATTY ACIDS AND OVERALL COMPOSITION.—The fatty acids (table 4) of the triglycerides in *K. elegans* oil are typical for a Myristicaceae in that myristic acid is the principal component (3). Capillary column gc indicated that the C_{18} unsaturated acids were oleic, linoleic and α-linolenic.

Gc analysis of a silylated sample of the intact oil is given in table 5. Identifications were based on retention data gathered from isolated or partially purified samples that had previously been identified by gc-ms. Quantitation is given in area percentages to allow estimation of the composition, although gc responses for the various compounds were not determined.

TABLE 4. Fatty acid composition of *Knema elegans* oil (as methyl esters).

Component*	Area % by gc
14:0	71.0
16:0	6.7
18:0	1.6
18:1†	16.0
18:2†	2.1
18:3†	0.1
20:0	1.9

*Chain length: number of double bonds.

†Capillary column gc shows these esters to have the retention characteristics of 18:1 (9), 18:2 (9, 12) and 18:3 (9, 12, 15).

TABLE 5. Composition of silylated *Knema elegans* oil by gc.

Area, %	RRT[a]	Identity of parent compound[b]
1.9	0.03	14:0
0.3	0.05	16:0
1.2	0.09	18:1
Trace	0.10	Cardanol **3b** ?
Trace	0.14	20:0
0.3	0.15	Cardanol **3d**+**3e**
0.4	0.16	Anacardic **1a**
0.4	0.18	Cardanol **3f**+**3g**
3.8	0.23	Anacardic **1b**
7.2	0.24	Galbacin **4**
1.9	0.25	Resorcinol **2b**
1.4	0.29	Cardanol **3h**
11.4	0.31	Anacardic **1d**+**1e**+Lignan **5**
1.2	0.32	Anacardic **1c**
0.8	0.33	Cardanol **3i**+Resorcinol **2d**+**2e**
5.3	0.35	Lignan **6**
10.6	0.40	Anacardic **1h**
1.4	0.41	Anacardic **1f**+**1g**
0.7	0.43	Resorcinol **2j**
0.5	0.46	Resorcinol **2i**
12.1	0.47	Anacardic **1j**
9.7	0.49	Anacardic **1i**
1.6	0.60–0.90	Diglycerides
0.7	0.93	?
0.2	0.98	?
17.8	1.00	C_{42} triglyceride
0.4	1.02	?
4.1	1.04	C_{44} triglyceride
2.3	1.10	C_{46} triglyceride
0.3	1.15	C_{48} triglyceride
Trace	1.20	C_{50} triglyceride
Trace	1.24	C_{52} triglyceride
Trace	1.30	C_{54} triglyceride

[a]Relative retention time, C_{24} triglyceride = 1.00.

[b]Except for galbacin (**4**) and the triglycerides, which do not silylate.

EXPERIMENTAL

SAMPLE PREPARATION.—Oil was extracted from finely ground seed with petrol (bp 35–60°). A BF_3/MeOH-catalyzed preparation from the intact oil gave methyl esters of the normal fatty acids. Acetylated methyl esters of anacardic acids were prepared by acetylation in acetic anhydride-pyridine (2:1) followed by methylation with CH_2N_2. The unsaturated anacardic esters were oxidized to diols by OsO_4 (13). All silylations were carried out in pyridine with hexamethyldisilane-trimethylchlorosilane (2:1).

SPECTROMETRY AND SPECTROSCOPY.—The gc-ms instruments and computerized data acquisition-reduction system have been previously described (14). The gc was equipped with a 122 x 0.32 cm glass column packed with 3% OV-1. Column operating conditions varied depending on the sample but were in the range of 200° to 280°.

Pmr spectra were obtained from $CDCl_3$ solutions with either a Varian XL-100 or a Bruker WH-90 instrument; chemical shifts were measured relative to tetramethylsilane. Ir spectra were recorded from $CHCl_3$ solutions with a Perkin-Elmer model 137 spectrophotometer.

CHROMATOGRAPHY.—Preliminary separation of *K. elegans* oil was accomplished on a 1-cm i.d. silica column, which was sequentially eluted with: 300 ml of hexane-ether (95:5), 200 ml of hexane-ether (85:15), 200 ml of hexane-ether (50:50) and 300 ml of ether. Fifty-milliliter fractions were collected, and the progress of the chromatography was monitored by tlc on silica with a hexane-ether (75:25) solvent system.

Gc of methyl esters of normal fatty acids has been described (15). This preparation was also analyzed on a 25-m glass capillary column coated with SP-1000 (Packard Instrument Co.) operated at 200°. For gc of the intact oil (after silylation), a 1 m X 0.32 i.d. glass column packed with 3% OV-1 (Applied Science Labs.) was temperature programmed from 180° to 360° at 2°/min.

A reverse-phase column (Magnum-9, ODS-2, Whatman, Inc.) was used for hplc in a Waters ALC-201 (Waters Assoc.) instrument equipped with a differential refractometer. Precoated silica plates (Brinkman) were used for tlc (0.25-mm layers) and plc (2-mm layers). Solvent systems are given, as appropriate, below.

ISOLATION OF COMPOUNDS.—Anacardic acids were eluted from the silica column in fractions 3–7. Individual compounds (as acetylated methyl esters) were isolated by hplc with CH_3CN or with methanol-water (9:1) solvent systems.

Fractions 4 and 5 from the silica column contained galbacin (**4**), which was purified by hplc (CH_3CN–H_2O, 80:20) and, finally, crystallized from ethanol. Mp 115–116° (uncorr.); $[\alpha]^{26}D$ −106°; ms: 340, M^+ (42), 190 (100), 178 (19), 162 (29), 160 (10), 149 (11), 145 (37), 135 (9), 131 (6), 117 (13), 108 (8), 91 (8); pmr (90 MHz $CDCl_3$) δ1.05 (6H, *d*), δ1.77 (2H, *m*), δ4.63 (2H, *d*), 5.95 (4H, *s*), δ6.80–6.92 (6H, *m*). Compound **5** was found in fraction 12 from the silica column and was purified by plc with chloroform-ether (75:25). Pmr (100 MH_3, $CDCl_3$) δ1.05 *d*, 1.70 *m*, 3.89 *s*, 4.60 *d*, 5.92 *s*, 6.76–6.90 *m*; ms of TMS derivative: 414, M^+ (25), 399 (5), 264 (18), 236 (14), 234 (12), 190 (100), 175 (27), 162 (10), 160 (10), 149 (10), 145 (31), 135 (11), 117 (15), 73 (37). Lignan **6** was eluted in fraction 14 from the silica column. Pmr (100 MHz, $CDCl_3$) δ1.05 *d*, δ1.65 *m*, δ3.86 *s*, δ4.65 *d*, δ6.82–6.94 *m*; ms of TMS derivative: 488, M^+ (28), 473 (9), 264 (100), 252 (21), 249 (26), 236 (33), 233 (48), 205 (10), 179 (8), 73 (42).

A series of related compounds (structure **2**) were isolated by plc from fraction 13 (silica column) ms of TMS derivatives—**2b**: 436, M^+ (24), 421 (5), 310 (5), 281 (16), 268 (100), 253 (5), 237 (3), 193 (3), 147 (12), 93 (6), 75 (15), 73 (52); **2d(e)**: 462, M^+ (20), 447 (3), 310 (5), 281 (15), 268 (100), 253 (6), 193 (3), 147 (10), 93 (3), 73 (65); **2h**: 470, M^+ (27), 455 (4), 310 (4), 281 (13), 268 (100), 253 (7), 237 (3), 193 (3), 147 (11), 104 (5), 91 (50), 73 (95); **2j**: 496, M^+ (25), 481 (4), 405 (7), 310 (4), 281 (15), 268 (100), 239 (2), 193 (3), 147 (8), 104 (7), 91 (37), 73 (82); **2i**: 498, M^+ (19), 483 (3), 310 (4), 281 (13), 268 (100), 253 (7), 193 (2), 147 (8), 104 (6), 91 (44), 73 (83).

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