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6-(PENTADEC-8-ENYL)-2,4-DIHYDROXYBENZOIC ACID FROM SEEDS OF *GINKGO BILOBA*

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Key Word Index—*Ginkgo biloba*; Ginkgoaceae; phenolic lipids; 6-alkylresorcylic acids; 4-hydroxyanacardic acids; 6-(pentadec-8-enyl)-2,4-dihydroxybenzoic acid; 6-tridecyl-2,4-dihydroxybenzoic acid; structural determination.

Abstract—Novel phenolic lipids isolated from *Ginkgo biloba* were identified as 6-(pentadec-8-enyl)resorcylic (97%) and 6-tridecylresorcylic (3%) acids. They are 4-hydroxyanacardic acids and represent the postulated precursors of cardols (5-alkylresorcinols).

INTRODUCTION

Lipids extracted from *Ginkgo biloba* or from the Anacardiaceae contain anacardic (6-alkylsalicylic) acids, cardanols (3-alkylphenols) and cardols (5-alkylresorcinols) where alkyl is *n*-C_{13} to *n*-C_{19} with 0 to 3 double bonds [1–5]. The combination of aliphatic fatty acid chains with a phenolic moiety makes these compounds of interest in many respects. They are of industrial importance as major components of cashew nut shell oil. Anacardic acids have antibiotic properties [6,7] and urushiols (3-alklycatechols) are the toxic principle of poison ivy [8–10]. A polyketide mechanism had been suggested for the biosynthesis of these phenolic lipids [11,12].

The lipids of immature ginkgo seeds contain up to 75% anacardic acids and a study of their biosynthesis showed that the salicylic moiety is formed from acetate but, more efficiently, from malonate [13,14]. The accompanying cardanols most likely are derived from anacardic acids by biological decarboxylation [15]. Corresponding 6-alkylresorcylic acids have been postulated [15] as precursors of cardols but hitherto they have not been found in natural materials. In this paper we describe the isolation and identification of these acids from ginkgo seed lipids.

RESULTS AND DISCUSSION

The new 6-alkylresorcylic acids occur, like anacardic acids, in ginkgo as free acids. By TLC or PLC of the lipids extracted from ginkgo seed, a fraction was isolated (5% total lipids) which, like the anacardic acids (70%), exhibited blue fluorescence in UV light but migrated slower. The UV and PMR spectra of this fraction indicated the presence of aromatic and long-chain alkyl structures. The material behaved in reactions with CH_2N_2 very similar to anacardic acids [16] and under mild reaction conditions one Me group was introduced selectively while under more stringent conditions, a total of three Me groups were added. MS of the major component and of several derivatives showed a M^+ which were in agreement with a pentadecenyl-dihydroxybenzoic acid. By inference from anacardic acids, the mono-Me derivative is a dihydroxy Me ester and the tri-Me derivative is the dimethoxy Me ester.

The Me esters of the dihydroxy acids and their di-Me ethers have the same R_f on TLC, but they can be distinguished by the brownish–purple coloration of the former with $FeCl_3$ [17] (the latter do not respond to this the reagent). The same distinction is observed with Me anacardates and their methyl ethers. However, anacardates migrate faster on TLC than their ethers. In the latter, hydrogen bonding to the carbonyl of the ester group is eliminated and, thereby, adsorption is increased. In the dihydroxy esters this effect of one OH group apparently is cancelled by the decrease in adsorption caused by the etherification of the second OH group which is not hydrogen bonded. Such interpretation is supported by the R_f of 6-methyl-2,4-dihydroxybenzoic (orsellinic) acid, its Me ester, 4-methyl ether Me ester and dimethyl ether Me ester.

IR spectra of the dihydroxy acids and their mono- and tri-Me derivatives were consistent with such a structure. The UV spectrum of the acids showed the same λ_{max} as orsellinic acid and the PMR spectrum of the trimethylated acids was in conformance with the pertinent features shown by di-Me ethyl orsellinate. In particular, both compounds show a singlet at δ 6.3 for 2 aromatic protons. This rules out an *ortho* position of the protons where strong spin-spin coupling would be expected, and implicitly rules out a 3- or 5-hydroxyanacardic structure for the novel acids.

As with anacardic acids, only the fully methylated dihydroxy acids are amenable to GLC which revealed two components in amounts of 97 and 3%. GC–MS gave a M^+ which were in agreement with pentadecenyl- and tridecylresorcylic acids, respectively. Ozonization–hydrogenation of the mixture yielded heptaldehyde as the only aliphatic fragment. Therefore, the olefinic bond of the pentadecenyl moiety is in position ω7. A *cis*-configuration can be assigned to this double bond since IR did not reveal absorption at 970 cm^{-1} which is characteristic of a *trans* double bond. PMR showed a signal at δ 5.3 which is in agreement with the assignment of a *cis* structure.

It is concluded that the new acids are 4-hydroxyanacardic acids, namely 6-(pentadec-8-enyl)resorcylic acid which is accompanied by a small amount of the corresponding tridecyl acid. The acids are associated in ginkgo seeds with cardols and had been predicted as precursors of the latter which would derive from them by decarboxylation [15]. Acids of the type described here have been chemically synthesized [18] and have been postulated as precursors also in the biosynthesis of 2-methyl-5-alkylresorcinols which recently have been found in cashew nut shell oil [19].

It is reasonable to assume that 4-hydroxyanacardic acids are synthesized in the plant by a polyketide pathway as are the anacardic acids [13,14]. Incubations of acetate [-^{14}C] with immature ginkgo seeds led to 4-hydroxyanacardic acids of average activity, 60 μCi/mM, and to anacardic acids, 47 μCi/mM. These activities are rather similar when compared to 190 μCi/mM of fatty acids from lipids of the same incubation. It seems likely that 4-hydroxyanacardic and anacardic acids share the initial synthetic system but the oxygen in position 4 is not removed when 4-hydroxyanacardic acids are produced.

EXPERIMENTAL

Immature seeds of *Ginkgo biloba* were harvested in early July at the University of Northern Iowa, Cedar Falls, IA. Lipids were extracted by homogenization with $CHCl_3$–MeOH (2:1) and recovered as described in ref. [16]. About 10 g crude lipids was obtained from 250 g fresh seeds.

Isolation of 4-hydroxyanacardic acids. Total lipids were fractionated by PLC in portions of 70 mg/plate on Si gel H, 1 mm thick with $CHCl_3$–MeOH–10 M NH_4OH, 65:30:4, as solvent. Hydroxyanacardic acids were detected by their blue UV fluorescence in a band below that of anacardic acids. They were extracted from the adsorbent with Et_2O satd with conc HCl. After filtration through sintered glass, the soln was washed to neutrality with H_2O, and the Et_2O was then removed under vac. The hydroxyanacardic acids were further purified by PLC in hexane–Et_2O–HOAc, 75:25:1, and recovered as described before.

Methylation. Procedures were similar to those described for methylation of anacardic acids [16]. Hydroxyanacardic acids were dissolved in Et_2O containing 10% MeOH and exposed to CH_2N_2 carried by a stream of N_2 bubbling through the soln [20]. The treatment with CH_2N_2 was interrupted as soon as a faint yellow color persisted in the soln. MS of the product (direct probe) showed that only the carboxyl group was methylated. The fully methylated compounds were obtained by pouring Et_2O containing CH_2N_2 in large excess onto the acids and then adding MeOH to 20% vol. CH_2N_2 was replenished after 2 hr. The trimethylated product was obtained after 5 hr and purified from polymethylene by filtration and PLC. It was recovered from Si gel by extraction with $CHCl_3$. Orsellinic acid was synthesized [21–23] and subjected to the same procedures. In this case, the prolonged reaction with CH_2N_2 was incomplete and the tri-Me derivative had to be separated from the 4-methyl ether of methyl orsellinate [24]. However, the latter was converted into tri-Me orsellinate by further treatment with CH_2N_2 in Et_2O catalyzed by MeOH.

TLC. Si gel H was used and the following R_f values, in hexane–Et_2O–HOAc, 75:25:1, were found: 4-hydroxyanacardic acids, 0.21; Me esters, 0.31; di-Me ether Me esters, 0.30; anacardic acids, 0.47; Me esters, 0.66; Me ether Me esters, 0.46; R_f values in hexane–Et_2O–HOAc, 30:20:1, orsellinic acid, 0.39; Me ester, 0.42; 4-Me ether Me ester, 0.69; di-Me ether Me ester, 0.38.

GLC of trimethylated 4-hydroxyanacardic acids. The derivatives were chromatographed in a 0.3 × 122 cm aluminium column, with 5% OV-1 on Gas Chrom Q at 225° with He at an inlet pressure of 2.7 atm and FID. ECL in reference to methylated saturated anacardic acids (6-tridecylsalicylic = 13.0; 6-pentadecylsalicylic acid = 15.0) were 6-tridecylresorcylic acid, 15.1; 6-(pentadec-8-enyl)-salicylic acid, 14.7; 6-(pentadec-8-enyl) resorcylic acid, 16.8. For both resorcylic type acids the increment caused by the additional OMe group is close to 2.1.

Ozonization. Trimethyl 4-hydroxyanacardates. (1 mg) were dissolved in 3 ml pentane and exposed to a stream of O_3 in O_2 [25] at −60° for 15 sec. After rapid removal of O_3 by a stream of N_2, commercial Lindlar catalyst was added and H_2 bubbled through the soln for 10 min. Catalyst was filtered off and the soln concentrated to about 0.1 ml vol. Aliquots of this were used for GLC on DEGS at 110° which gave one peak corresponding to that of heptaldehyde. Under conditions described for GLC of trimethyl hydroxyanacardates, a minor peak appeared which corresponded to trimethyl tridecyl resorcylic acid and a major peak which may be assigned to the multi-functional aldehyde arising with heptaldehyde from the pentadec-8-enyl compound.

Spectrometry. UV λ_{max}^{EtOH} nm, 4-hydroxyanacardic acids: 257 and 294; anacardic acids, 240 and 301; orsellinic acid, 253 and 292. IR ν_{max} cm^{-1}, hydroxyanacardic acids (CCl_4 and CS_2): 3510 (OH), 2868 (CH), 1623 (CO_2H), 1463 (CH), 1262 (CO_2H), 1225 (OH), 900 (CO_2H), 724 (CH_2); Me ester (liquid film): 3335 (OH), 2878 (CH), 1650 (CO_2Me), 1615, 1585 (Ar), 1440 (CH), 1257 (CO_2Me), 1197 (OH), 1105 (CO_2Me), 720 (CH_2); di-Me ether Me ester (liquid film): 2873 (CH), 1715 (CO_2Me), 1599, 1582 (Ar), 1460 (CH), 1260 (CO_2Me), 1200 (C–O–Me), 1095 (CO_2Me), 1073 (C–O–Me), 719 (CH_2). PMR (Fourier transform spectrometer, 79.54 MHz, $CDCl_3$) δ, tri-Me hydroxyanacardates: 0.9 (3H, *t*, terminal Me), 1.3 (–CH_2– envelope), 2.0 (4H, *d*, 2x CH=CH–C$\underline{H}_2$), 2.5 (2H, *t*, $\underline{H}_2$C–Ar), 3.8 (6H, *s*, 2x H_3C–O–Ar), 3.9 (3H, *s*, H_3 COOC), 5.3 (2H. *t*, HC=CH), 6.3 (2H, *s*, 2x H–Ar); di-Me ethyl orsellinate: 1.4 (3H, *t*, $\underline{H}_3$C–CH_2–OOC), 2.3 (3H, *s*, H_3C–Ar), 3.8 (6H, *s*, 2x H_3C–O–Ar), 4.4 (2H, *q*, H_3C–C$\underline{H}_2$–OOC), 6.3 (2H, *s*, 2x H–Ar); 4-Me Et orsellinate, 1.4 (3H, *t*, $\underline{H}_3$C–CH_2OOC), 2.5 (3H, *s*, H_3C–Ar), 3.8 (3H, *s*, H_3C–O), 4.4 (2H, *q*, H_3C–C$\underline{H}_3$–OOC), 6.3 (2H, *m*, 2x H–Ar), 11.8 (1H, *s*, HO–Ar). MS. 70 eV *m/e* (rel. int.). 6-(pentadec-8-enyl)resorcylic acid: 362 (0.7, M^+); Me ester: 376 (14.5, M^+), 358 (1.1, $[M–H_2O]^+$), 344 (5.1, $[M–MeOH]^+$), 316 (1.6, $[M–HCO_2Me]^+$), 182 (100, $[M–C_{14}H_{26}]^+$), 150 (24.9, $[M–C_{14}H_{26}–MeOH]^+$); di-Me ether Me ester: 404 (12.9, M^+), 123 (9.7, $[M–C_{14}H_{26}–CO_2Me]^+$); di-TMSi ether (by TMCS) Me ester: 520 (15.1, M^+); di-TMSi ether TMSi ester: 578 (2.4, M^+); di-TMSi ether Me ester of 6-tridecylresorcylic acid: 494 (19.3, M^+); di-TMSi ether TMSi ester: 552 (2.8, M^+).

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