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Prostaglandin Synthetase Inhibitors from the African Medicinal Plant Ozoroa mucronata

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Two prostaglandin synthetase inhibitory anacardic acids were isolated from O. mucronata. The structures of these inhibitors were established by spectroscopic means. Efficient syntheses of these two via directive metallation were reported.

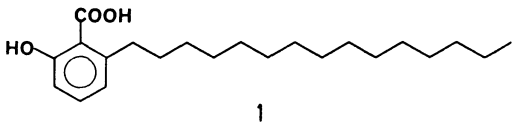
Prostaglandins (PGs) have been found in many insects.[1] Although the role of these compounds playing in the metabolism of insects is thought to be quite complex, PGE_2 has recently been implicated in the reproductive cycle of the cricket Teleogryllus commodus. The presence of PGE_2 in the spermatheca of female T. commodus is known to cause increased oviposition, and it is now believed that a PG-synthetase complex is transferred from the male's spermatophore to the female's spermatheca during mating whereupon PGE_2 is then biosynthesized within the female.[2] The above finding prompted us to investigate PG-synthetase inhibitors from plant sources for their possible use as insect control agents. We report here the isolation of two potent PG-synthetase inhibitors from the root of the East African medicinal plant Ozoroa mucronata (Anacardiaceae). The hot water extract of the root is drunk to elicit spontaneous abortion, and to cure gonorrhea, diarrhea, intestine parasites, and stomach troubles.[3]

We found the methanol extract of the root bark to exhibit PG-synthetase inhibtory activity. This extract (100 g) was separated into hexane, methylene chloride, ethyl acetate, and water soluble portions, and the portions were reexamined using a PG-synthetase inhibitory assay. The methylene chloride soluble portion was observed to retain the biological activity and was submitted to further separation using silica gel column chromatography with benzene-ethyl acetate (100:1, v/v) as a solvent mixture. The active portion (450 mg), shown to be two compounds by reverse phase HPLC, was then subjected to low pressure column chromatography using ODS-silica gel with acetonitrile-methanol-acetic acid (200:100:0.15, v/v) as a solvent system. This resulted in the isolation of two compounds active in the bioassay in 0.2% (1), mp 89-89.5 °C, and 0.1% (2) yields.

The structures of compounds 1 and 2 were determined by spectroscopic means. The IR spectra of both 1 and 2 exhibited bands typical of an aromatic ring (1600,

1450 cm^{-1}) and a carboxylic acid group (2800-3200 and 1670 cm^{-1}). Compound 1 exhibited ^{1}H NMR resonances typical of a saturated hydrocarbon chain at δ 2.97 ppm (t, 2H, Ar-CH_2-), 1.12-1.59 ppm (m, 26H, -CH_2-) and 0.86 ppm (t, 3H, -CH_3). The presence of the chain was confirmed by the related ^{13}C NMR shifts at δ 36.46 (t, 1C, Ar-CH_2-), 32.01-22.63 (t, 13C, -CH_2-) and 14.10 ppm (q, 1C, -CH_3). Three aromatic proton resonances were also observed, which was consistent with the IR data. The ^{13}C NMR spectrum displayed 6 carbon resonances characteristic of phenyl ring at δ 163.58 (s, C-2), 147.81 (s, C-6), 135.39 (d, C-4), 122.75 (d, C-5), 115.45 (d, C-3), 110.45 ppm (s, C-1), and a carboxylic acid carbonyl at δ 175.95 ppm. The resonance at 175.95 ppm appeared to be shifted up-field due to conjugation with the aromatic ring. The down-field shift of one of the ring carbons (163.58 ppm) indicated it to be a site of hydroxylation. The up-field shift of the ring carbon (110.45 ppm) was used to assign the hydroxyl group ortho to the carboxyl group on the basis of the 12.7 ppm deshielding effect of ortho hydroxyl groups.[4] Substituent effects could not be used to assign the relative position of the side chain because of the small induced shifts predicted by this type of substituent. However, the analysis thus far suggested that 1 was related to anacardic acids. These compounds are known to be found among plants of the Anacardiaceae.[5] Comparison of the ^{13}C NMR data of 1 to closely related compounds in the literature[6] place the side chain at C-6 and identified as 6-(pentadecyl)salicylic acid.

The ^{1}H and ^{13}C NMR spectra of 1 and 2 were very similar. Thus 2 differed from 1 only in that the side chain of 2 is unsaturated (^{1}H NMR; 5.33 ppm, m, 2H, -CH=CH-: ^{13}C NMR; 129.87 and 129.83 ppm, -CH=CH-). The location of the double bond in the side chain was assigned to C-10' and C-11' based on products resulting from ozonization. The stereochemistry was determined to be cis from the diagnostic shifts of the two allylic methylene carbons (26.30 and 27.18 ppm), as these would have appeared at 33 ppm if the bond were trans.[7] Thus, the structure of 2 was established as 6-[10'(Z)-pentadecenyl]salicylic acid.[8]



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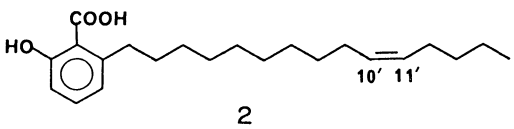

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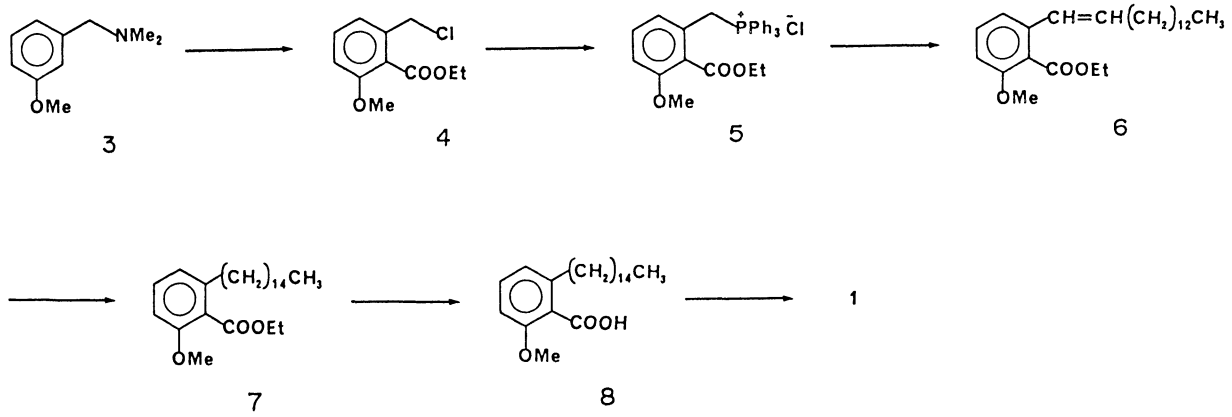
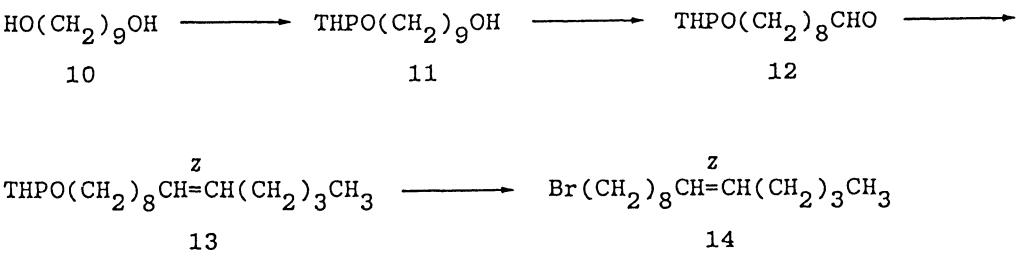
Anacardic acid ($C_{15:0}$),[9] 1, was prepared as outlined below. The directive metallation of 3-methoxy-N,N-dimethylbenzylamine (3) with butyllithium under Rapoport's conditions[10] gave 2-lithio-3-methoxy-N,N-dimethylbenzylamine. Quenching the lithio derivative with a large excess of ethyl chloroformate gave the chloride (4) in 67% yield. The compound 4 proved to be a versatile intermediate for the synthesis of 6-alkyl- or 6-alkenylsalicylic acids and 6-alkenylsalicylic aldehyde, for example, pyriculol.[11] The phosphonium salt (5), which was obtained from 4 and triphenylphosphine, was treated with lithium bis(trimethylsilyl)amide[12] at -78 °C and quenched with tetradecanal to give a 1:3 mixture of cis and trans olefins (6) in 50% yield. Catalytic hydrogenation of 6 using 5% platinum on carbon gave (7).

Prolonged alkaline hydrolysis in refluxing aqueous DMSO[13] gave the salicylic acid derivative (8) in good yield. The final demethylation by boron tribromide[14] gave anacardic acid ($C_{15:0}$), mp 90.2-91.5 °C (from hexane), which was identical with natural product by direct comparison.

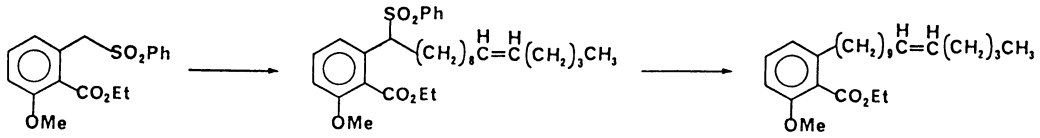


Anacardic acid ($C_{15:1}$),[9] 2, was prepared by the alkylation of 6-(phenylsulfonyl)methylsalicylate (9) which was obtained from the chloride 4 and benzenesulfinic acid sodium salt in DMF.[15] The necessary alkyl bromide (14) was prepared according to the following scheme.



Partial protection of 1,9-nonanediol with 1 equivalent of dihydropyran and pyridinium p-toluenesulfonate[16] gave the monoalcohol (11) in 60% yield. Oxidation of 11 by Swern's procedure[17] gave the aldehyde (12) in 92% yield. Wittig olefination of 12 with pentyltriphenylphosphonium iodide and potassium t-butoxide in THF at room temperature gave the cis olefin (13) in 72% yield.[18] Hydrolysis of the THP ether followed by tosylation and bromination gave the bromide (14) in 57% yield from 13. Treatment of the sulfone 9 with LDA at -68 °C followed by quenching with the bromide 14 gave the alkylated product (15) in 64% yield. The benzenesulfonyl group was removed by reduction of 15 with 5% sodium amalgam[19] to give the ester (16) in 88% yield. The two protecting groups were removed by successive treatment with sodium hydroxide in refluxing aqueous DMSO[13] and with sodium ethanethiolate in DMF[20] to give anacardic acid ($C_{15:1}$), 2, mp 45.8-46.2 °C, which was identical with natural product (TLC, IR, and NMR).

In conclusion, we have found a more versatile pathway for construction of 6-alkenylsalicylic acids.



9 15 16

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8) More detailed bioassays with pure compounds showed that the oviposition rate of female T. commodus mated with males treated with 3 ng of 1 laid significantly fewer eggs. Antimicrobial activity was also observed against several Gram positive bacteria at concentrations of 10 to 50 $\mu g/cm^3$.
9) The suffixes indicate the number of carbons and degree of unsaturation in the side chain, respectively.
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