Pergamon

0031-9422(94)00842-6

Phytochemistry, Vol. 39, No. 2, pp. 405–407, 1995

0031-9422/95 $9.50 + 0.00

ANACARDOSIDE FROM THE SEEDS OF *SEMECARPUS ANACARDIUM*

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(*Received in revised form* 22 *September* 1994)

Key Word Index—*Semecarpus anacardium*; Anacardiaceae; phenolic glycoside; anacardoside; structure determination; 2D NMR techniques.

Abstract—From the seeds of *Semecarpus anacardium*, a new phenolic glucoside, anacardoside, was isolated, and its structure and configuration were elucidated by a combination of NMR techniques as 1-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyloxy-3-hydroxy-5-methylbenzene.

INTRODUCTION

The fruits of *Semecarpus anacardium* are claimed to be useful in treating leprosy, rheumatoid arthritis, piles, asthma, cough, sexually transmitted diseases such as syphilis and gonorrhea, and skin diseases such as leucoderma [1, 2]. The seeds are eaten in certain parts of India and are considered nutritious. Several Ayurvedic preparations such as "Bhallataka rasayana", "Amritha bhallataki", "Brihat bhallataka lehya" are marketed in India. The fruits of *S. anacardium* are subjected to a purification process before they are used for Ayurvedic medicines because they are considered to be toxic. Purified fruits are claimed to possess rejuvenating properties, increasing longevity, bringing a glow to the face, a sweetness in tone and improvement in vision [3].

A literature survey revealed that no chemical investigations had been carried out on the seeds of *S. anacardium*, while the nut shells had been investigated extensively. Anacardic acid [4], semicarpol, bhilawanol [5], monolefin I, diolefin II [6, 7], bhilawanol-A and bhilawanol-B [8] have been reported from the vesicant oil of the nuts. The biflavonoid amentoflavone was isolated from the leaves [9], and several other biflavonoids like biflavone A_1, A_2, B and C [8], tetrahydroamentoflavone, tetrahydrobustaflavone [9], jeediflavanone, semecarpuflavanone, galluflavanone and anacarduflavone [10–13] were isolated from the nut shells. The fruits of *S. anacardium* have been reported to possess anti-cancer [14–18], anti-inflammatory, anti-arthritic and anthelmintic activities [19, 20].

In this publication we present the isolation, structure elucidation and unambiguous NMR assignments of the new phenolic glycoside anacardoside (**1**) using ^{1}H NMR, ^{13}C NMR (BB and DEPT), COSY, HETCOR, HOHAHA and selective INEPT experiments [21–23].

RESULTS AND DISCUSSION

Anacardoside (**1**) gave a negative reaction with acetic anhydride–H_2SO_4 for a steroid, but, gave a positive Mollisch test for sugars and $FeCl_3$ test for phenols. The positive HR-FAB mass spectrum indicated a molecular formula of $C_{19}H_{28}O_{12}$. The ^{1}H NMR spectrum (Table 1) showed three broad singlets at δ7.15, 6.72 and 6.68, which show long-range coupling to each other in the COSY spectrum. These signals, together with a three-proton singlet at δ2.15, suggested the presence of a 3,5-dihydroxytoluene moiety. We also observed the presence of two anomeric proton resonances at δ5.44 (*d*, J = 7.5 Hz) and 5.05 (*d*, J = 7.5 Hz), and several carbinol signals between δ3.7–4.7, indicating the presence of a disaccharide moiety linked to the aromatic group. The ^{13}C NMR and DEPT spectra of **1** (Table 1), showed the presence of six sp^2 carbon signals (three quaternary and three methine), two anomeric carbon signals, 10 aliphatic carbinol carbon signals (eight methine and two methylene) and one methyl carbon signal.

A combination of COSY, NOESY, HOHAHA, HETCOR and selective INEPT experiments led to the unambiguous assignment of all proton and carbon NMR signals. The signals of the anomeric protons were used as starting points in the COSY spectrum, and allowed the assignment of all proton signals. The vicinal coupling constants involving H-1, H-2, H-3, H-4 and H-5 (Table 1) of the sugar moieties indicated the β configuration of the anomeric carbon and permitted identification of both moieties as glucopyranosides. The multiplicities of most of the signals, except for H-3″ and H-4″, could be deter-

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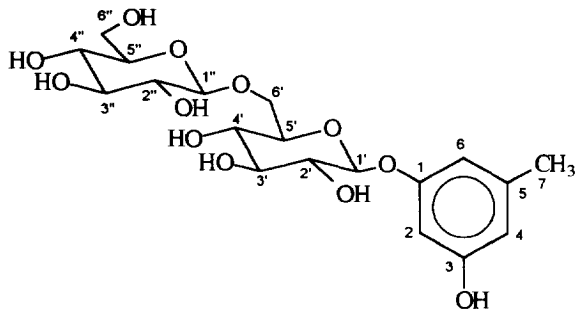

Anacardoside (1)

Table 1. ^1H and ^{13}C NMR of anacardoside (1) (pyridine-d_5, ^1H: 500 MHz; ^{13}C: 90.8 MHz)

	^1H	^{13}C
1	—	159.5
2	7.15 (*br s*)	101.8
3	—	159.5
4	6.68 (*br s*)	110.7
5	—	140.1
6	6.72 (*br s*)	108.7
7	2.15 (*s*)	21.3
1′	5.44 (*d*, 7.5)	102.0
2′	4.15 (*dd*, 9, 7.5)	74.4
3′	4.23 (*t*, 9)	77.8
4′	4.19 (*t*, 9)	70.5
5′	4.10 (*ddd*, 9, 6, 2)	77.2
6′a	4.66 (*dd*, 12, 2)	69.1
6′b	4.29 (*dd*, 12, 6)	
1″	5.05 (*d*, 7.5)	104.7
2″	4.01 (*dd*, 8.5, 7.5)	74.9
3″	4.18 obsc.*	77.7
4″	4.17 obsc.	71.2
5″	3.78 (*ddd*, 8.5, 6, 2)	77.9
6″a	4.41 (*dd*, 11.5, 2)	62.1
6″b	4.27 (*dd*, 11.5, 6)	

*Obscured.

Coupling constants (J in Hz) in parentheses.

mined, and the HOHAHA experiment was critical in the assignment of H-3′ and H-3″ through the relayed cross-correlation peaks between H-1′ and H-3′, and H-1″ and H-3″. The signals for H-3′, H-3″, H-4′ and H-4″ appear in a very congested area of the ^1H NMR spectrum, making their assignment very difficult, by COSY alone; the HOHAHA experiment was therefore important. The $1 \rightarrow 6$ linkage of the glucose moieties was clearly indicated by the shift to lower fields observed for C-6′ (δ69.1). This $-CH_2OH$ group normally appears at *ca* δ 62 when the OH group is free [24]. The signals of those carbons bearing hydrogen were assigned by a HETCOR experiment.

A series of selective INEPT experiments led to the assignment of the quaternary carbons and also confirmed the connectivities of the two sugars and of the sugar to the aromatic ring (Table 2). Irradiation of H-1″ at δ5.05 enhanced the signal corresponding to C-6′ (δ69.1), confirming the $1 \rightarrow 6$ linkage between the glucose units. The irradiation of the anomeric proton H-1′ at δ5.44 enhanced the signal of the quaternary aromatic carbon at δ159.5 (C-1) confirming the connection of the sugar fragment to the aromatic ring. This irradiation also permitted differentiation of the carbon signals C-1 and C-3, which appear close ($\Delta\delta$ = 0.06 ppm) in the spectrum. Irradiation of the methyl group H_3-7 enhanced C-4 (δ110.7), C-5 (δ140.1) and C-6 (δ108.7), confirming the connection of the methyl group to the aromatic ring. The remaining irradiations listed in Table 2 substantiated the structure and confirmed the assignments leading to the structure of anacardoside as **1**, i.e. orcinol 1-gentiobioside.

EXPERIMENTAL

General. The ^1H NMR, COSY, HETCOR and HOHAHA spectra were recorded at 500.12 MHz; ^{13}C NMR and DEPT spectra, and selective INEPT experiments were recorded at 90.8 MHz. For the selective INEPT experiments, data sets of 16K covering a spectral width of 10 kHz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 ($^1{}^rJ$ = 6.7 Hz) in a 5-mm NMR tube [25]. The radio frequency field strength for the soft pulse was of the order of 25 Hz. For the aromatic protons $^3J_{C-H}$ = 7 Hz, for aliphatic protons $^3J_{C-H}$ = 5 Hz. IR spectra were recorded in a KBr pellet. Mps: uncorr.

chased from the local market and identified by Dr V. S. Raju, Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal, India. A voucher specimen (AVN-SA-93) is deposited in the University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India.

Extraction and separation. Fruits of *S. anacardium* (4 kg) were cracked open and seeds sepd from shells. The seeds (600 g) were powdered and extracted with C_6H_6, $CHCl_3$ and EtOH successively (cold maceration). The alcoholic extract (30 g), after evapn, was CC over silica gel (500 g). Elution with $CHCl_3$–MeOH (23:2) afforded a solid after recrystallization from $CHCl_3$ identified as sitosterol glucoside (220 mg, 0.04%). From the solvent obtained by elution with $CHCl_3$–MeOH (41:9) sucrose pptd (2 g, 0.33%). The mother liquor was concd and flash CC (silica gel 60 10–40μ, EtOAc–MeOH–H_2O, 81:11:8). Frs 30–80 afforded anacardoside (**1**) (300 mg, 0.05%).

Anacardoside (**1**). Crystalline solid, mp 117–119°; $[\alpha]_D$ −46° (MeOH; *c* 0.8); UV λ^{MeOH} nm (log ε): 203 (4.0), 220 (3.3), 250 (2.9), 256 (3.0), 261 (2.9); IR ν^{CHCl_3} cm^{-1}: 3371, 2921, 1598, 1457, 1322, 1163, 1071, 907, 838; ^1H and ^{13}C NMR: Table 1; positive FAB-MS *m/z* (rel. int.): 471 [M + Na] (12.4), 449 [M + 1] (8.0), 325 (2.2), 287 (2.9),

Table 2. Selective INEPT experiments on anacardoside (**1**) (pyridine-d_5, 90.8 MHz)

Proton irradiated	δ (ppm)	Carbon enhanced
2	7.15	159.5 (C-1), 159.5 (C-3), 110.7 (C-4), 108.7 (C-6)
6	6.72	159.5 (C-1), 110.7 (C-4), 101.8 (C-2)
4	6.68	159.5 (C-1),* 159.5 (C-3), 108.7 (C-6), 101.8 (C-2), 21.3 (C-7)
7	2.15	140.1 (C-5), 110.7 (C-4), 108.7 (C-6)
1′	5.44	159.5 (C-1)
1″	5.05	69.1 (C-6′)
6′	4.66	104.7 (C-1″), 70.5 (C-4′)
6″	4.41	71.2 (C-4″), 77.9 (C-5″)
2″	4.01	104.7 (C-1″), 71.2 (C-4″)
5″	3.78	77.7 (C-3″), 71.2 (C-4″), 104.7 (C-1″)

*Enhancement due to partial irradiation of H-6 at δ6.72.

163 (1.0), 124 (10.9), 115 (100); positive HR-FAB-MS *m/z*: found 449.1662 calc. for $C_{19}H_{29}O_{12}$ [M + 1], 449.1659.

Bioassay. Anacardoside (**1**) showed no activity in several cancer cell lines nor in the HIV-1 reverse transcriptase assay [26].

Acknowledgements—A.V.N. wishes to thank IFS, Sweden for financial assistance (Grant No. F/1689-1). Additional support was provided, in part, by a grant from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. We thank Dr J. M. Pezzuto for biological tests, the Research Resources Center at UIC for the provision of NMR spectroscopic facilities and Mr R. Dvorak for the HR-FAB spectrum. Thanks also to Dr V. S. Raju for identification of plant material, Mr N. Reddy for collection of plant material and Mr Ramu, Mr Ramesh, Mr Prasad and Mr Mosaiah for help in separation of seeds from nut shells. M.R. wishes to thank Dr C. Mullangi and Mr S. Mullangi for their encouragement and invaluable help. R.R.G. wishes to thank CONICET — Argentina for a post-doctoral fellowship.

REFERENCES

1. Nardkarni, K. M. (1976) *Indian Materia Medica*, Vol. 1, 3rd edn, pp. 1119–1125. Popular Prakashan.
2. Kirthikar, K. R. and Basu, B. D. (1933) *Indian Medical Plants*, 2nd edn, pp. 666–667. Basu, L. M., Allahabad.
3. Sreenivasacharyulu, V. (1931) *Yogarathnakaram*, Vol. 1, p. 177. Swathantra Press, Nellore, A.P., India.
4. Chatopadhyaya, M. K. and Khare, R. L. (1969) *Ind. J. Pharm.* **13**, 104.
5. Pillay, P. P. and Siddiqui, S. (1931) *J. Ind. Chem. Soc.* **3**, 517.
6. Prakasa Rao, M. S. and Ramachandra Rao, L. (1970) *Current Sciences* **39**, 207.
7. Govindachary, T. R., Joshi, B. S., Kamal, V. N. and Vishwanathan, M. R. (1971) *Ind. J. Chem.* **9**, 1044.
8. Prakasa Rao, M. S., Ramachandra Rao, L. and Brown, R. T. (1973) *Phytochemistry* **12**, 671.
9. Ishratullah, K. H., Ansari, W. H., Rahman, W., Okigawa, M. and Kawan, N. (1977) *Ind. J. Chem.* **15B**, 615.
10. Murthy, S. S. N. (1983) *Phytochemistry* **22**, 1167.
11. Murthy, S. S. N. (1983) *Phytochemistry* **22**, 1518.
12. Murthy, S. S. N. (1983) *Phytochemistry* **22**, 2636.
13. Murthy, S. S. N. (1992) *Chim. Acta Turcica*, *V* **20**, 33.
14. Sunanda, V., Gotoskar, S. V. and Kamal, J. R. (1971) *Ind. J. Exp. Biology* **9**, 372.
15. Chitnis, M. P., Bhatia, K. G., Pathak, M. K. and Kesava Rao, K. V. (1980) *Ind. J. Exp. Biol.* **18**, 6.
16. Pathak, M. K., Ambaye, R. Y., Indap, M. A. and Bhatia, K. G. (1983) *Ind. J. Physiol. Pharmacol.* **27**, 166.
17. Indap, M. A., Ambaye, R. Y. and Gokhale, S. V. (1983) *Ind. J. Physiol. Pharmacol.* **27**, 83.
18. Ragini, V., Sankaranarayan, A., Chaudary, R. R., Mathur, V. S. and Chkravarthi, R. N. (1983) *Ind. J. Med. Res.* **77**, 902.
19. Satyavathi, G. V., Prasad, D. N., Das, P. K. and Singh, H. D. (1969) *Ind. J. Physiol. Pharmacol.* **13**, 37.
20. Kesava Rao, K. V., Gothoskar, S. V., Chitnis, M. P. and Kamal, J. R. (1973) *Ind. J. Physiol. Pharmacol.* **23**, 115.
21. Bax, A. J. (1984) *J. Magn. Reson.* **57**, 314.
22. Cordell, G. A. (1991) *Phytochem. Anal.* **2**, 49.
23. Cordell, G. A. and Kinghorn, A. D. (1991) *Tetrahedron* **47**, 3521.
24. Agrawal, P. K. (1992) *Phytochemistry* **31**, 3307.
25. Bax, A. (1983) *J. Magn. Reson.* **52**, 76.
26. Tan, G. T., Pezzuto, J. M., Kinghorn, A. D. and Hughes, S. H. (1991) *J. Nat. Prod.* **54**, 143.