SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF SOME ANACARDIC COMPOUNDS

M.V.R. Koteswara Rao, M.L. Sarma, K. Sridharan, N.A.N. Rao and P.K. Vijayaraghavan

Defence Food Research Laboratory, Mysore

(*Received* 25 *January* 1966)

A new series of anacardic compounds from cashewnut shell liquid phenol, anacardol, have been synthesised and screened for antibacterial and antifungal activity. All the anacardic compounds have been found active against *S. Typhosa* in dilutions varying from 1 : 10,000 to 1 : 1,000,000. The only compound found active against *A. Niger* is chloro-mercuri-tetrahydroanacardol.

Long chain *m*-alkenylphenols of the type anacardol (IA) and anacardic acid (IB) possess high bactericidal activity[1,2]. They are derived from cashewnut shell liquid (CNSL) *anacardiaceae*[3]. The oil itself possesses bactericidal, fungicidal and pesticidal activity[4-6]. The hydrogenated cashewnut shell liquid is a co-solvent for rotenone insecticides[7]. Chlorinated oil is a pesticide containing predominantly pentachloroanacardol. It is also used for the control of termites in wood[8]. Quarternary nitrogen compounds derived from tetrahydroanacardol (II A) are powerful germicides[9].

Anacardol has been shown to be active against *staphylococus aureus* in dilutions of 1 : 200,000 to 1 : 2,000,000. Anacardic acid and its sodium salts are also shown to be active in similar dilutions. The phenol co-efficient of disodium anacardate is reported to be 1·07 and 4·0 against *salmonella typhosa* and *staphylococus aureus* respectively[10]. By virtue of the long carbon chain, it possesses good surface activity and hence it is a bacteriostatic surfactant too. Quarternary nitrogen compounds from tetrahydroanacardol are, in general, water soluble and hence exhibit bacteriostatic activity in dilutions around 1 : 2,000,000. Nitrated anacardols have been reported to be fungicides[11]. This communication reports the synthesis of a new series of anacardic compounds and their antibacterial and antifungal activity.

SYNTHESIS OF ANACARDIC COMPOUNDS

The compounds are synthesised according to the scheme shown in Chart 1. Tetrahydroanacardoxyacetic acid (III) was prepared according to the procedure of Gandhi & Venkataraman[3] with slight modifications. It exhibits IR bands at 3350 cm^{-1} (hydroxyl), 1750 cm^{-1} (acid carbonyl) 1500-1600 cm^{-1} (benzenoid). Esterification of this with methanolic hydrogen chloride furnished the methyl ester (IV). Ethanolic hydrogen chloride converted tetrahydro-anacardoxyacetic acid into its ethyl ester (V) which displays IR bands at 3500 cm^{-1} (overtone of carbonyl), 1750 cm^{-1}(ester carbonyl), 1575-1600 cm^{-1} (benzenoid). Reduction of ethyl tetrahydroanacardoxyacetate with lithium aluminium hydride in ether afforded tetrahydro-anacardoxyethanol (VI), possessing IR absorption at 3700, 3400 cm^{-1}(hydroxyl), 1575-1600 cm^{-1}(benzenoid). Three moles of this alcohol and one mole of phosphorous trichloride combined to give tris—(2-tetrahydro anacardoxyethyl) phosphite (VII). Tetrahydroanacardol similarly furnished tris-tetrahydroanacardoxy phosphite (VIII). Chloromercuration of tetrahydroanacardol under similar conditions as reported in the case of phenol[12] resulted in chloromercuric—tetrahydroanacardol (XIV)

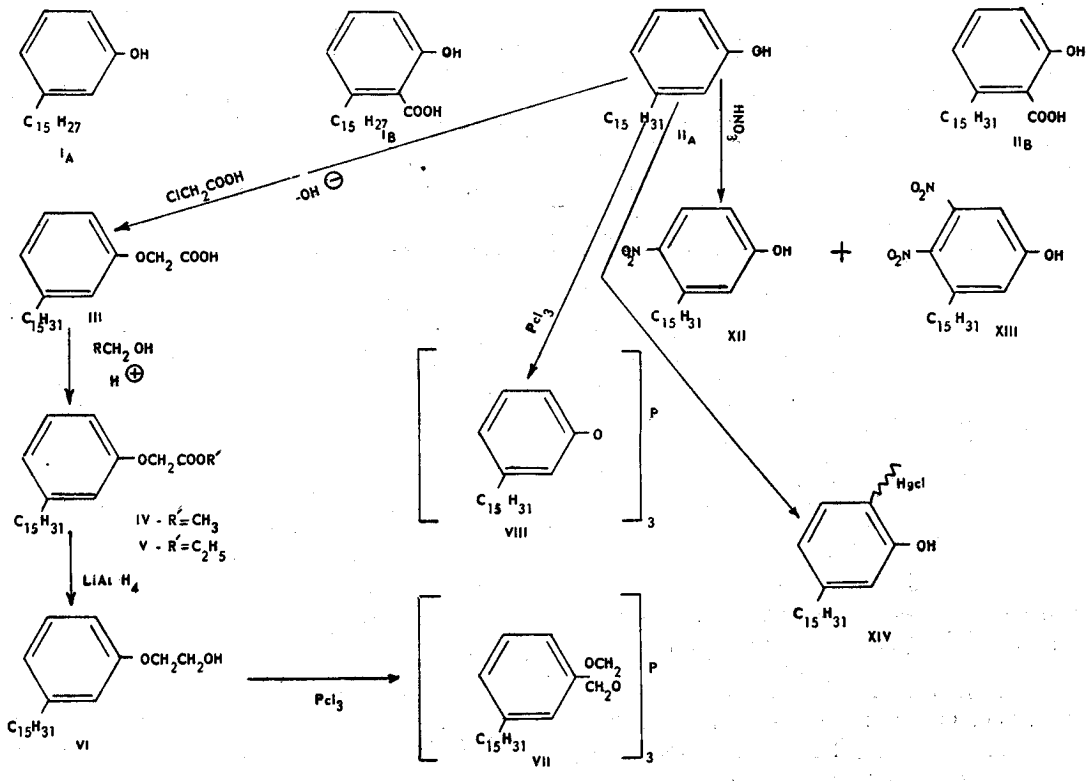

Chart 1.

In analogy with the above, the position of—*HgCl* was presumed to be ortho on the less hindered side of the hydroxyl group. 4-Nitro-5-pentadecyl phenol (XII) and 3, 4-dinitro 5-pentadecyl phenol (XIII) were prepared by the usual nitration of tetrahydroanacardol. The physical and chemical properties of these anacardic compounds are recorded in Table 1.

EXPERIMENTAL PROCEDURE

Tetrahydroanacardoxyacetic acid (III)—A homogeneous mixture of tetrahydroanacardol (3·0 gm), potassium hydroxide (1·32gm), water (2 ml), ethanol (6 ml), and chloroacetic acid (0·95gm) was refluxed on a steam bath for 4 hours. Towards the end, alcohol was distilled off, the residue acidified with diluted hydrochloric acid and extracted with ether. The sodium salt of tetrahydroanacardoxyacetic acid was thrown out on shaking the ether extract with 2 per cent aqueous sodium carbonate. It was collected at the pump on Whatman 41 filter paper, suspended in water (100 ml) and acidified with dil. hydrochloric acid.

Methyl tetrahydroanacardoxyacetate (IV)—The above acid (0·45gm) and saturated methanolic hydrogen chloride (50 ml) were refluxed on a steam bath for 4 hours. At the end, methanol was flashed off and water (50 ml) added. The mixture was saturated with sodium chloride and products taken in ether. Removal of the acid by extraction with 2 per cent sodium carbonate followed by distillation of solvent afforded methyl tetrahydroanacardoxyacetate as a viscous liquid. It formed long pale yellow needles from methanol.

TABLE I

PHYSICAL PROPERTIES OF ANACARDIC COMPOUNDS

Compound	Yield (%)	MP °C	Solvent of crystallisation	Elemental Analysis						Infrared bands (cms^{-1})
				Calculated			Found			
				C	H	P	C	H	P	
Tetrahydroanacardoxyacetic acid (III)	86	101—2	ethanol	76·25	10·50	..	76·23	10·61	..	3350 (hydroxyl) 1750 (acid carbonyl) 1600 (benzenoid)
Methyl tetrahydroanacardoxy acetate (IV)	90	42—44	methanol	76·60	10·64	..	76·21	10·62	..	
Ethyl tetrahydroanacardoxy acetate (V)	86	34—36	methanol	76·91	10·76	..	76·56	10·72	..	3500 (carbony overtone) 1750 (ester carbonyl) 1575—1600 (benzenoid)
Tetrahydroanacardoxy-enthanol (VI)	70	49	methanol	79·30	11·49	..	78·90	11·24	..	3700, 3375 (hydroxyl) 1575—1600 (benzenoid)
Tris—(2—tetrahydro-anacardoxyethyl)—Phosphite (VII)	84	43	Petroleum ether	..	..	2·89	..	..	2·25	..
Tris—tetrahydro-anacardoxy phosphite (VIII)	95	32	Petroluem ether	..	..	3·40	..	..	3·76	..
4—Nitro—5—pentadecylphenol (XII)	30	34	ethanol	..	..	..	..	..	..	..
3, 4—Dinitro—5—pentadecylphenol (XIII)	40	63	Petroleum ether	..	..	..	..	..	..	..
Chloromercuric tetrahydroanacardol (XIV)	55	38—41	Water	..	..	..	..	..	..	..

Ethyl tetrahydroanacardoxyacetate (V)—This was prepared from tetrachloroanacardoxyacetic acid in a similar manner as described above using ethanolic hydrogen chloride as long pale yellow needles from methanol.

Tetrahydroanacardoxyethanol (VI)—Ethyl tetra-hyrdoanacardoxyacetate (1·0 gm) in dry ether (10 ml) was slowly added to a slurry of lithium aluminium hydride (0·200 gm) in dry ether (10 ml) at 16°C. The mixture was thoroughly stirred for 30 minutes and left overnight. Excess lithium aluminium hydride was destroyed with ethyl acetate and the complex was decomposed with 10 per cent solution of Rochelle's salt. The product from ether was recovered after distillation to afford long colourless needles from methanol.

TABLE 2

BACTERIOSTATIC AND FUNGISTATIC ACTIVITY OF ANACARDIC COMPOUNDS

Compound No.	Nomenclature	Bacteriostatic Activity		Fungistatic Activity
		Active Dilution against *Salmonella typhosa*	Phenol coefficient	Diameter of zone of Inhibition of *Aspergillus Niger* on Sabouraud's Agar media
II A	Tetrahydroanacardol	1 : 1,000,000	..	*Nil*
II B	Tetrahydroanacardic acid	1 : 1,000,000	..	..
III	Tetrahydroanacardoxyacetic acid	1 : 1,000,000	..	..
IV	Methyl tetrahydroanacardoxyaceate	1 : 1,000,000 delayed growth observed in 1 : 10,000	..	..
V	Ethyl tetrahydroanacardoxyacetate	1 : 1,000,000	..	..
VI	Tetrahydroanacardoxyethanol	1 : 1,000,000	..	..
VII	Tris—(2—tetrahydroanacardoxyethyl)—phosphite	1 : 100,000	..	..
VIII	Tris—tetrahydroanacardoxy phosphite	1 : 100,000	..	..
IX	Potassium salt of IIA	..	Determination difficult due to sparing water solubility.	..
X	Potassium salt of IIB	..	1·01	..
XI	Potassium salt of III	..	4·20	..
XII	4—Nitro-5-pentadecylphenol	1 : 100,000	..	..
XIII	3, 4—Dinitro—5—Pentadecylphenol	1 : 1,000,000	..	..
XIV	Chloromercuri tetrahydroanacardol	1 : 1,000,000	..	6 cm
XV	Control (Acetone)	Complete growth	..	..
XVI	Standard (Mercapto-benzothiazole)	..	..	2 cm

Tris—2—tetrahydroanacardoxyethyl—phosphite (VII)—Phosphorous trichloride (0·05 ml) in benzene (10 ml) was slowly added to a mixture of tetrahydroanacardoxyethanol (0·5gm) and pyridine (0·15 ml) at 16°C. The contents were shaken for 30 minutes and left over-night. The pyridine hydrochloride was formed; it was filtered and the solvent distilled off. The residue was taken in xylene (20 ml) and refluxed over sodium (0·5 gm). The solution was cooled, filtered and distillation of xylene afforded the pale brown phosphite ester.

Tris-tetrahydroanacardoxy phosphite (VIII)—This was prepared from tetrahydroanacardol (1·0 gm) in a similar manner as described above. It is a pale brown solid.

Chloromercuric tetrahydroanacardol (XIV)—Mercuric acetate (1·0 gm) was slowly added to tetrahydroanacardol (1·54 gm) at 170°C and the clear solution was poured into hot water. The mixture was boiled for 5 minutes and filtered. The filtrate was again brought to boiling, treated with sodium chloride (0·20 gm), filtered, and on cooling afforded colourless crystals of chloromercuric tetrahydroanacardol.

ANTIBACTERIAL ACTIVITY

Serial dilution method[13]—The substances (IIA-XIV) were dissolved in acetone or water depending on their solubility. 1 ml of 1 : 100; 1 : 1,000; 1 : 10,000; 1 : 100,000 and 1:1,000,000 dilution of each was added aseptically to 9 ml of sterile nutrient broth (peptone 10gm, beef extract 10gm and sodium chloride 5g in one litre distilled water; *pH* 7·2—7·4 after autoclaving). The tubes were then inoculated with 24 hour broth culture of *salmonella typhosa* and incubated at 37°C for 48 hours. These were then subcultured into fresh nutrient broth and incubated for 24 hours at 37°C and observed for growth. A control test with 1 ml of acetone (solvent for the compounds) was also carried out simultaneously.

Determination of phenol co-efficients[14]—The phenol coefficients of compounds X and XI were determined by the well known Riedel-Walker method using a culture of *Salmonella typhosa*.

ANTIFUNGAL ACTIVITY[15]

Sterile Sabouraud's agar medium (dextrose, agar, peptone, 40 : 20 : 10 dissolved in 1 litre, *pH* 5·2—5·6 after autoclaving) was melted and cooled to 45°C and poured aseptically into sterile 9 cm petri dishes. After the agar hardened, the entire surface of each plate was inoculated with *Asperglus Niger* by means of a dry sterile cotton swab. At the centre of the plate a circular hole (1 cm dia) was cut and a few drops of 10 per cent solution of the test sample in alcohol were filled into it. The plates were then incubated for 5 days at room temperature (27—30°C); a clear zone of inhibition around the circular hole indicated the effectiveness of the antifungal compound. In Table 2 are given the results of antibacterial and antifungal activity of the various compounds tested.

RESULTS AND DISCUSSION

As seen from Table 2, all the anacardic compounds have shown considerable antibacterial activity even in dilutions as low as 1 : 1, 000,000. The determination of phenol coefficients of sodium salts of compounds I, II and III presented considerable difficulty due to their sparingly soluble nature in water; however, the potassium salts, *viz.* compounds X and XI, being completely water soluble, facilitated the determination of phenol coefficients. The phenol coefficient of compound IX, although a potassium salt, could not be determined as it is sparingly soluble in water. The high phenol coefficient of compound XI, as compared to compound X may be attributed to the presence of anacardoxyacetic acid moiety in its structure.

With the exception of compound XIV, all the anacardic compounds listed above, failed to show antifungal activity against *A. Niger*. The high antifungal activity exhibited by compund XIV (Zone of inhibition 6 cm, as compared to 2 cm in the case of standard) could be attributed to the presence of chlorine and mercury in its structure.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Professor D. K. Banerjee and Dr. B. H. Iyer, Indian Institute of Science, Bangalore for the reports of microanalysis and IR Spectra; to Dr. M. Sirsi for supplying a culture of *A. Niger* and to the Scientific Adviser to the Minister of Defence for permission to publish this work. One of us (M. V. R. Koteswara Rao) is thankful to the Council of Scientific and Industrial Research, New Delhi for the offer of a Pool Officer's post.

REFERENCES

1. EICHBAUM, F. W., HAUPHMANN, H. & ROTHSCHILD, H., *Anais, Assoc. Quim. Brasil*, **4** (1945), 83 ; *Chem Abstrs.*, (1946), 6443.
2. EICHBAUM, F. W., *Mem. Inst. Butantan*, **19** (1946), 71; *Chem. Abstrs.* (1947), 5969.
3. GANDHI, R. C. & VENKATARAMAN, K., *J. Indian, Chem. Soc.*, **5** (1942), 89.
4. HEPBURN, J. S., BOERICKE, G. W., RICKETS, R. & BOONA, E. D., *J. Amer. Inst. Homeopathy*, **43** (1950), 73; *Chem. Abstrs.* (1951), 9748.
5. LEOS, A. T. & EICHBAUM, F. W., *Mem. Inst. Buntantan*, **20** (1947), 13 ; *Chem. Abstrs.* (1948), 6932.
6. RAO, B. C. S., & SETHI, C. S., Indian Patent No. 69,621 (to Council of Scientific and Industrial Research, New Delhi), 1962.
7. HARVEY, M. T., U. S. PATENT No. 2, 368, 709 (to Harvel Corpn, New Jersey), 1945.
8. WALCOTT, G. N., *J. Econ. Entomology*, **42** (1949), 273.
9. GULATI, A. S., KRISHNAMACHAR, V. S. & RAO, B. C. S., *Indian J. Chem.*, **2** (1964), 114.
10. BISWAS, A. K. & ROY, A. B., *Nature*, **182** (1958), 1299.
11. DAWSON, C. R. & WASSERMANN, D., U. S. PATENT No. 2, 502, 708 (to Harvel Corpn., New Jersey), 1950.
12. WHITMORE, F. C. & HANSON, E.R., "ORAGNIC SYNTHESIS COLL. Vol. 1" (John Wiley and Sons, London), 1961, p. 161.
13. MC. KEE, C. M., RAKE, C. D. & MENZEL, A. F., *J. Immunology*, **48** (1944), 259.
14. "*Methods of Analysis*" (Association of Official Agriculture Chemists, Washington) 1960, p. 63.
15. "Encyclopaedia of Chemical Technology, Vol. II (Interscience Publishers, New York) 1948, 102.