Mem. Inst. Butantan,
19:69-134, 1946.

BIOLOGICAL PROPERTIES OF ANACARDIC ACID (O-PENTADECADIENYL-SALICYLIC ACID) AND RELATED COMPOUNDS

BY F. W. EICHBAUM

(From the Department of Microbiology of the Escola Paulista de Medicina and the Department of Immunology of the Instituto Butantan, S. Paulo, Brazil)

PART I

GENERAL DISCUSSION — BACTERICIDAL ACTION

The isolation of antibiotic substances from molds and bacteria has also stimulated the study of higher plants in the same direction (Osborn, Lucas & Lewis). As Dubos points out, the production of these antibiotic substances is by no means an exclusive attribute of microorganisms, but is widely distributed in the animal and plant kingdoms. Until the industrial development of synthetic chemotherapeutical products, during the last decades, the plants furnished the bulk of all pharmaceutical preparations, and amongst them we find chemotherapeutically very active products such as quinine, emetine, chaulmoogra oil and others. Apart from the latter substance, whose mode of action is still under discussion, no substance with systemic antibacterial action has so far been isolated from higher plants (*). On the other hand, products with local antibacterial action, such as some ethereal oils and balms, belong to the most ancient therapeutic treasure of mankind.

In October 1943, we obtained a sample of Cashew nut oil, the fruit shell oil of the tropical tree Anacardium occidentale, in order to study its therapeutic action on spontaneous and artificial wounds in rabbits. Cashew nut oil (**) has been known for a long time as a skin irritant and vesiculant which provokes

---

(*) Therefore, it is misleading to call an antibacterial substance "penicillinlike", unless it displays also a definite chemotherapeutic activity cf. the Editorial in the J. Amer. Med. Ass. "Penicillinlike substances from higher plants"; in fact, not a single substance is mentioned that might claim to be really "Penicillinlike".

(**) Sometimes called also Cardol; in reality, Cardol is only one of the two main constituents of Cashew nut oil.

Received for publication in 16/2/946.

the formation of blisters, similar to those produced by cantharidin; it has been used, occasionaly, for the treatment of warts, psoriasis, eczemas and for the external treatment of leprotic lesions (Hansen & Looft; Lima; Furtado; Morais; Lacerda).

The alcoholic tincture is recommended as a vermifuge. Exact clinical observations on the therapeutic value of cashew nut oil are practically non-existent and the main significance of this product lies, to-day, in its ample technical application in the lacquer and varnish industry. The favorable therapeutical results we obtained in the treatment of infected skin wounds in rabbits, by application of a 5% ointment of Cashew nut oil, led us to test the bactericidal activity of this substance also in-vitro. It could be shown that alcoholic solutions of the oil, emulsified by slow addition of water, possessed in fact a very strong germicidal power against the common pyogenic bacteria. The next step was to isolate the two main constituents of cashew nut oil, anacardic acid and cardol (*), which were described for the first time by *Staedeler* as early as 1846; both substances proved to be about equally active with regard to their bactericidal power.

Anacardic acid (o-pentadecadienyl-salicylic acid) which makes up about 90% of the crude cashew nutshell liquid, contains a non-saturated alkyl with 15 carbon atoms linked to a salicylic acid radical.

Cardol and anacardol, which share the remaining 10%, contain the same alkyl group linked to resorcinol and phenol, respectively.

Apart from anacardic acid and Cardol (impure) the following sub-products have been studied:

Anacardol
Tetrahydro-anacardic acid
Tetrabrom-anacardic acid
Tetrachlor-anacardic acid
Isomeres of bromation on the ring of anacardic acid
Acetyl-anacardic acid (acetylester of anacardic acid)
Acetyl tetrahydro-anacardic acid
Methylether of Sodium anacardate (2 metroxy-6 pentadecadienyl-benzoic acid)
Methylether of Sodium tetrahydro-anacardate
Dimethylanacardic acid (2 metoxy-6 pentadecadienyl-methylbenzoate)

(*) The fraction called here *Cardol*, which remains dissolved in the alcoholic solution after precipitation of anacardic acid by lead hydroxide, proved in a later analysis to consist — in greater part at least — of Anacardol.

Dimethyl-tetrahydro-anacardic acid (2 metoxy-6 pentadecylmethylbenzoate)
Ethyl anacardate
Sodium anacardate
Sodium tetrahydro-anacardate
Ammonium anacardate
Copper anacardate
Silver anacardate
Copulation product of anacardic acid and sulfanilamide

(cf. Table No. V)

(pag. 89/90)

The alkali salts of anacardic acid can be classified amongst the anionic detergents. Substances of this group have been synthetized recently on a very large scale (*); apart from their technical usefulness they possess a well defined germicidal power, which is directd mainly against gram-positive germs.

The special interest we attribute to anacardic acid and its subproducts seems justified by the fact that these easily obtainable substances of vegetal origin display some qualitative and quantitative features that distinguish them from most other anionic detergents.

The alkali salts of anacardic acid belong to the most potent anionic detergents; they possess, in particular, a staphylococcidal activity equalled by few others. Their bactericidal activity is not only limited to gram-positive germs, but includes also gram-negative genera such as Neisseria, Brucella, Pasteurella, Hemophilus and amongst the Enterobacteriaceae, the proteus bacilli. Their activity is practically independent of the actual pH of the medium. The alkali salts possess good solubility in lipid solvents and fair solubility in water; consequently, their germicidal activity becomes patent in watery as well as in oily solutions, although to a lesser degree in the latter. Finally, anacardic acid possesses an antipyretic activity, probably due to its salicylic nucleus, and a vermicidal action, both phenomena so far unknown in other detergents.

The study of anacardic acid and related products has been extended to the following problems:

1. germicidal action
2. detoxifying action
3. antienzymatic action
4. anthelmintic action
5. anti-ectoparasitic action

(*) Among the synthetic anionic detergents, alkyl compounds with 15 C atoms are o be found only exceptionally.

6. larvicidal action
7. antiprotozoic action
8. antifebrile action
9. toxic action
10. clinical application.

GERMICIDAL ACTION

Methods — The antibacterial power of anacardic acid against various bacteria can be checked grossly by measuring the diameter of the germfree zone, formed round a central hole in an agar dish which is filled with a 1% Na anac (*) solution (cf. photo No. I and II).

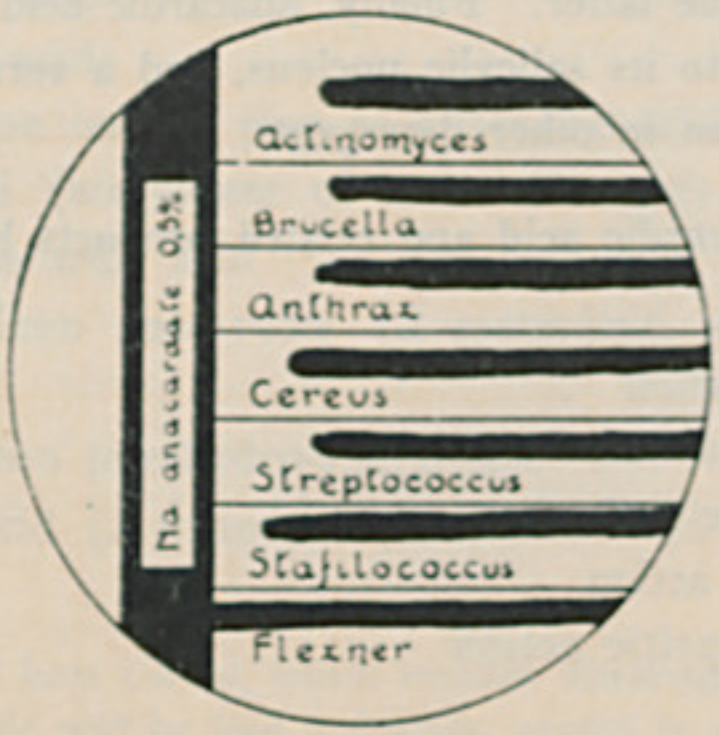
Alternatively, another technique, as advocated by Flemming, gives a still better impression of the relative sensitiveness of various germs towards Na anac:

The method consists in cutting out a strip of agar from a culture plate and planting various bacteria in streeks at right angles to the gutter thus formed; the gutter has been previously filled with a mixture of equal parts of liquefied agar and a 1% Na anac solution. The active substance thus diffuses into the agar and inhibits the growth of bacteria for a distance varying with their sensitivity to Na anac. When testing for gonococci and other delicate germs, the agar is prepared with 1/4 — 1/3 part of ascitic fluid or serum.

Special care should be taken that the quantity of the planted germs is approximately equal for all tested samples, since the size of the sterile zone depends to a certain degree on the density of the inoculum.

Results — Graph No. I, which condenses a great number of tests, illustrates this type of experiment, showing the absolute resistance of the Entereobacteriaceae, the relative sensitivity of Brucellae, Neisseriae, Pasteurellae and of various gram-positive bacteria.

GRAPH No. 1



(*) "Na anac.", in the following is used as an abreviation for Sodium anacardate.

This type of experiment, however, does not allow any conclusion as to whether the absence of bacterial growth is due to a bactericidal or a bacteriostatic process. We tried to settle this issue by comparing the antibacterial activity of various Na anac. dilutions in two parallel series. In the first series the agar was mixed with various Na anac. concentrations and, after solidification, inoculated with a given amount of staphylococci (bacteriostatic test: Table Ia). In the second series a suspension of staphylococci was incubated for 15' (at room temperature) with varying concentrations of Na anac. and was afterwards transfered to a Petri dish and mixed with liquefied agar (bactericidal test: Table Ib).

TABLE NO. IA AND IB

Ia. Bacteriostasis

To each Petri dish (containing 20 cc agar and 0.1 cc of the various dilutions of Na anac.) 0.1 cc of bacterial suspension (staphylococci) is added.

	Na anac.			
Initial dilution of Na anac.	Amount per Agar dish	Amount per 1 cc agar	No. of germs per cc after 24 hours' incubation	Degree of activity
1) 1/100	1 mg	50 γ	0	+++
2) 1/1000	100 γ	5 γ	33,000	±
3) 1/10000	10 γ	0.5 γ	150,000	—
4) 1/100000	1 γ	0.05 γ	150,000	—
5) 1/1000000 ...	0.1 γ	0.005 γ	150,000	—
6) Saline control	—	—	150,000	—

Ib. Bactericidia

1 cc of various dilutions of Na anac. is mixed with 1 cc of bacterial suspension (Staphylococci); after 15' contact, 0.2 cc are transfered into 20 cc agar.

Initial dilution of Na anac.	Amount of Na anac. per 1 cc mixture Na anac. + bacteria	Total amount of Na anac. per agar dish	Na anac.: Amount per 1 cc agar	No. of germs per cc after 24 hours' incubation	Degree of activity
1) 1/100	5 mg	1 mg	50 γ	0	+++
2) 1/1000	500 γ	100 γ	5 γ	0	+++
3) 1/10000	50 γ	10 γ	0.5 γ	0	+++
4) 1/100000	5 γ	1 γ	0.05 γ	925	+
5) 1/1000000 ..	0.5 γ	0.1 γ	0.005 γ	150,000	—
6) Saline control	—	—	—	150,000	—

Conclusion — Table Ia shows, that the limit of "bacteriostasis" lies at a concentration of 5γ/cc agar. In Table Ib., reduced growth is to be observed up to the fourth agar dish, which corresponds to a final Na anac concentration of 0.05γ/cc agar. Since this concentration, according to Table Ia., is inactive, the reduced growth in the fourth dish (Table Ib.) cannot be due to a bacteriostatic action; it was the 15 minutes' contact of 5γ Na anac that killed the greater part of the cocci — and this corresponds exactly to the value also found to be active in Table Ia. It follows, therefore, that the antibacterial activity of Na anac is not a bacteriostatic but a bactericidal one (*). This was also corroborated by experiments on animals: highly virulent hemolytic streptococci were brought in contact, during 15', with varying amounts of a Na anac solution (dilution 1/2000 — 1/200000) and afterwards injected, by intraperitoneal route, into mice; the animals injected with 0.5 cc of each "mixture" survived, whereas the controls died 24 — 48 hours after the inoculation.

No chemotherapeutic action of Na anacardate and related products (cf. Table No. V) on streptococci, pneumococci and anthrax bacilli could be observed. Neither did the intraperitoneal injection of Na anac solutions, 5 — 10' following an intraperitoneal injection of streptococci, prevent deadly bacteremia. (**)

In order to measure the sensitiveness of various germs towards Na anac by a more accurate method, the different bacterial species were tested in a "bactericidal" experiment:

A series of seven tubes was set up, each containing 1 cc of the bacterial suspension in test; the number of germs per cc amounted, on the average, to 200000 — 400000/cc; in a few instances smaller inocula (1000000 germs per cc) or denser inocula (200000 germs per cc) were used. Falling concentrations of Na anac (1/1000 — 1/10000000) in a 1 cc volume were added to subsequent tubes; the last (control) tube was completed with physiological saline solution. The final dilutions of Na anac were consequently 1/200; 1/2000; 1/20000 etc.

After 15' exposure to room temperature, 0.1cc was drawn from each tube and put into a Petri dish and mixed immediately with liquefied agar. Another

---

(*) In the same direction lie also the results obtained by varying the time of contact between anacardate and bacteria. The following table shows the number of living germs in a suspension of 200000 staphylococci per cc mixed with equal parts of a 1/100000 Na anac. solution, after varying periods of contact.

time of contact	No. of germs
5' - 10'	5000/cc
15'	450/cc
30'	10/cc
60'	0
Control 60'	100000/cc

(**) Experiments on mice.

Mem. Inst. Butantan,
19:69-134, 1946.

Action of Na anacardate on Actinomyces (the hole in the centre of the agar dish contains 0.2 cc of the Na anacardate solutions).

PHOTOGRAPH I
Activity of a 1/100 Na anacardate solution.

PHOTOGRAPH II
Activity of a 1/10000 Na anacardate solution.

0.1cc from each tube was transferred into 5 cc of glucose broth. After a 48 hours' incubation at 37°, readings were made both from agar and broth (*). In doubtful cases, a subculture was made from the glucose broth. The inoculation in broth proved to be, in general, more sensitive than the inoculation in agar. All tests were performed in duplicate, triplicate, or even more frequently.

TABLE No. II

Bactericidal power of sodium anacardate against various bacteria after 15' contact at room temperature

Concentration of Na anacardate

	1/200	1/2,000	1/20,000	1/200,000	1/2,000,000	1/20,000,000
Neisseria gonorrhoeae	0	0	0	+	+++	+++
Staphylococcus haemol. aur.	0	0	0	0 - +	+++	+++
Streptococ. pyogenes	0	0	0	0	± - +	+++
Bac. anthracis	0	0	0	± - +	+++	+++
Brucella melitensis	0	0	+	+++	+++	+++
Pasteurella aviseptica ...	0	0	±	+++	+++	+++
Mycobact. tuberculosis (human strain "Ratti") ..	0	0	0	+	+++	+++
Escherichia coli	+++	+++	+++	+++	+++	+++
Salmonella paratyphi B. (Schottmueller)	+++	+++	+++	+++	+++	+++
Salmonella typhi	+++	+++	+++	+++	+++	+++
Salmonella paratyphi B. (Breslau)	+++	+++	+++	+++	+++	+++
Bac. pyocyaneus	+++	+++	+++	+++	+++	+++
Bac. proteus (X 19)	0	±	+++	+++	+++	+++
Penicil. notatum	0	±	+++	+++	+++	+++
Aspergil. niger	++	+++	+++	+++	+++	+++
Yeast (strain isolated from the air)	±	+++	+++	+++	+++	+++

Readings: 0 no growth; ± isolated colonies; + weak growth; ++ medium growth; +++ full growth (equal to saline control).

(*) The growth of the tubercle bacilli cultures (in Sauton's medium) was checked after a 4 weeks' incubation at 37°.

With the exception of proteus bacilli and tubercle bacilli, which showed a different sensitiveness of various strains, the members of the other tested genera did not reveal any remarkable differences as to their sensitivity towards Na anac.

Conclusion — The foregoing Table II shows the high sensitiveness of all gram-positive bacteria toward Na anac, highest for streptococci, weaker for gram-negative genera such as Neisseria, Brucella, Pasteurella, and complete insensitiveness of the Enterobacteriaceae (except for the slightly sensitive proteus bacilli) as well as of the bac. pyocyaneus.

A suspension containing 600000 typhoid bacilli per cc, mixed with 1 cc of a 1/100 Na anac solution, became sterile only ofter 48 hours' contact. (The number of germs in the NaCl control had dropped, during the same time, to 100000 germs/cc); paratyphoid B bacilli behaved in the same way, whereas Shiga bacilli and coli bacilli survived more than 72 hours under the same conditions.

The behaviour of proteus bacilli varies according to the strain chosen. A 20 minutes' contact with a 1/100 Na anac solution kills practically all strains; a 1/1000 solution kills some strains; others suvive, but when subcultured in agar, generally only the 0 forms will develop; 2 cc of a 1/100 Na anac solution added to 20 cc agar in a Petri dish either inhibit the growth of proteus bacilli entirely, or make them grow in the O form, according to the sensitivity of the respective strains.

This observation agrees with the findings of Lominsky and Lendrum, who tested anionic, cationic and undissociable detergents as to their "antiswarming" activity; among 54 tested products, 42 proved to be active, some of them even in very high dilutions. There was a certain relation between surface activity and antiswarming power.

When a mixture of proteus bacilli (H X 19) and typhoid bacilli (Ty 2) is exposed for 15' at room temperature to the action of a 1/200 Na anac solution, the subculture gives only growth of typhoid bacilli in Agar and Teagues medium. As the same substance suppresses the growth of all gram-positive germs, it might serve as a useful device for the isolation of the Enterobacteriaceae. In order to eliminate with certainty even the more resistant proteus strains, the contact with the Na anac solution might be prolonged to 30 or even 60 minutes without impairing the viability of the other Enterobacteriaceae.

The critical bactericidal concentration of Na anac for tubercle bacilli lies at 5γ/cc, which kills the tubercle bacilli after a 30 minutes' contact; after a 15' contact the destruction is incomplete.

In his studies on the antibacterial action of Sodium ricinoleate (which we found about ten times weaker than Na anac) Larson obtained different results when inoculating solid or liquid media, a phenomenon he relates to the changed surface tension in the liquid media. Our experiments with anacardates in the same direction have not yet been performed on a sufficient scale to permit a definite conclusion. In liquid media we did not observe, either with Na anac or with ricinoleate, a submerged growth of the tubercle bacilli as described by Larson *et al.* It cannot be decided, for the moment, whether these contradictory results depend only on the strains used for the respective experiments.

The inoculation of a suspension of bovine tubercle bacilli (Vallée) which had remained in contact with a 1% Na anac solution for 15, 30 and 60 minutes respectively, did not produce tuberculosis in guinea pigs after 4 months of observation. In the same period, 3 out of 4 control animals showed a progressive tuberculosis, the fourth animal only a local process at the inoculated site.

The vegetative forms of anaerobic bacteria (Cl. tetani, Cl. perfringens, Cl. septicum) are readily killed even by high dilutions of Na anac; the spores of Cl. tetani and Cl. septicum survive even a prolonged contact (1 hour) with a 1/100 Na anac solution, whereas the spores of Cl. perfringens and aerobic bacilli (anthrax, subtilis) are very sensitive.

Molds and yeasts, with few exceptions, are very resistant. Penicillium notatum is only very slightly inhibited in an agar dish containing Na anac (cf. technique page 74).

The Spirochaeta gallinarum is highly sensitive to the action of Na anac: a 1/2000 dilution, mixed with equal parts of infected blood, immediately immobilizes the spirochaetes, in spite of the presence of proteic substances, which commonly reduce the antibacterial activity to a great extent. An anti-Flexner bacteriophage was not inactivated by a 2 hours' contact with a 1/1000 Na anac solution.

The sensitivity of motile bacteria to anacardic acid and derivatives, at various concentrations, can be rapidly tested by the hanging-drop method. The motility test gives results which practically parallel the bactericidal test. (Table III).

Motility test of B. proteus X19, Bac. subtilis, Salmonella Breslau

(in contact with different concentrations of Na anac. — Hanging drop method)

TABLE No. III

Concentration of Na anac.		Time of contact								
		0'	5'	10'	15'	20'	25'	30'	60'	120'
B. proteus H X 19	1/200	+	±	0	0	0	0	0	0	0
	1/2000	+	+	+	+	±	0	0	0	0
	1/20000	+	+	+	+	+	+	+	+	+
	Physiol. NaCl	+	+	+	+	+	+	+	+	+
Bac. subtilis	1/200	±	0	0	0	0	0	0	0	0
	1/2000	+	±	0	0	0	0	0	0	0
	1/20000	+	+	+	±	0	0	0	0	0
	Physiol. NaCl	+	+	+	+	+	+	+	+	+
Salmonella Breslau	1/200	+	+	+	+	+	+	+	+	+
	1/2000	+	+	+	+	+	+	+	+	+
	1/20000	+	+	+	+	+	+	+	+	+
	Physiol. NaCl	+	+	+	+	+	+	+	+	+

Readings: motile +
only few germs / weakly motile } ±
immobile 0

Cultures made after the same intervals gave the following result:

		Time of contact				
		0'	15'	30'	40'	60'
B. proteus H X 19						
Na anac.	1/200	4 colonies (0-form)	ø	7 colonies (0-form)	ø	ø
	1/2000 ...	9 colonies (0-form)	2 colonies (0-form)	ø	ø	ø
	1/20000 ..	"H" growth	"H" growth	"H" growth	"H" growth	"H" growth
NaCl control (*) ...		"H" growth	"H" growth	"H" growth	"H" growth	"H" growth
B. subtilis						
Na anac.	1/200	ø	ø	ø	ø	ø
	1/2000 ...	1% of the control	ø	ø	ø	ø
	1/20000 ..	15% of the control	ø	ø	ø	ø
NaCl control (*) ...		100%	100%	100%	100%	100%
Salmonella Breslau						
Na anac.	1/200 ... 1/20000 .	100% of the control				
NaCl control (*) ...		100%	100%	100%	100%	100%

Sterility = ø
(*) Number of germs approximately 300000/cc.

Influence of pH on the bactericidal activity of Na anac.

It has been shown that the bactericidal activity of anionic and cationic detergents is greatly influenced by the actual pH — the anionic detergents being most active at an acid, the cationic at an alkaline pH (Baker, Harrison and Miller). When testing the bactericidal activity of anacardic acid against staphylococci and anthrax bacilli at various pH steps, we were surprised to discover that the actual pH had apparently no influence on the bactericidal activity of anacardic acid (cf. Table IV).

TABLE No. IV

Influence of pH on the bactericidal effect of sodium anacardate at various concentrations

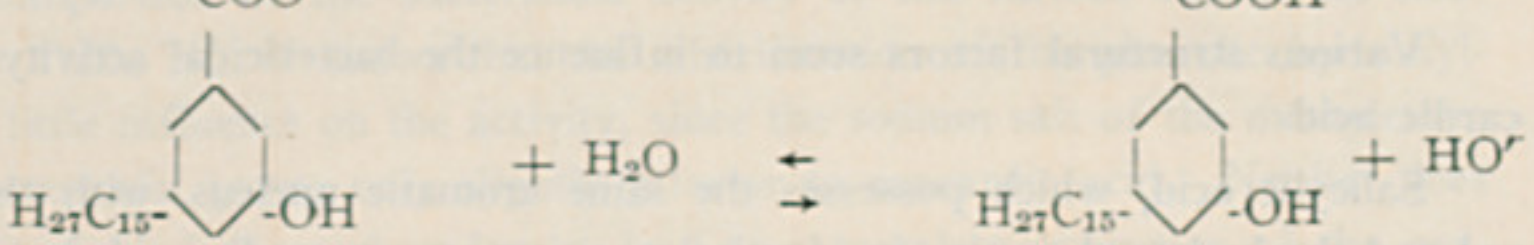
(The technique of this experiment corresponds to the "bactericidal experiment" in Table Ib.)

Tube No.	Buffer solution pH		Sodium anacardate 1/10000	Suspension of Staphylococci		No. of germs per 1 cc	
1	3.6	1 cc	1 cc	1 cc	15' minutes' contact at room temperature. Transference of 0.1 cc from each tube into a Petri dish, mixing immediately with melted agar. Incubation for 48 hours at 37°. Readings after 48 hours incubation.	0	The Buffer solutions pH 3.6; 4 and 5 were made up with an acetic acid — sodium acetate mixture, the buffer solutions pH 6 — pH 9 with a mono-potassium phosphate — Borax mixture, (cf. T. W. Kolthoff, "Säurebasen Indicatoren", Berlin, 1932, pp. 257/258).
2	4	1 cc	1 cc	1 cc		0	
3	5	1 cc	1 cc	1 cc		0	
4	6	1 cc	1 cc	1 cc		0	
5	7	1 cc	1 cc	1 cc		0	
6	8	1 cc	1 cc	1 cc		0	
7	9	1 cc	1 cc	1 cc		0	
			Sodium anacardate 1/100000				
8	3.6	1 cc	1 cc	1 cc		0	
9	4	1 cc	1 cc	1 cc		0	
10	5	1 cc	1 cc	1 cc		0	
11	6	1 cc	1 cc	1 cc		500	
12	7	1 cc	1 cc	1 cc		20	
13	8	1 cc	1 cc	1 cc		0	
14	9	1 cc	1 cc	1 cc		20	
			Sodium anacardate 1/1000000				
15	3.6	1 cc	1 cc	1 cc		0	
16	4	1 cc	1 cc	1 cc		8,000	
17	5	1 cc	1 cc	1 cc		200,000	
18	6	1 cc	1 cc	1 cc		210,000	
19	7	1 cc	1 cc	1 cc		280,000	
20	8	1 cc	1 cc	1 cc		270,000	
21	9	1 cc	1 cc	1 cc		220,000	
			Physiol. saline solution				
22	3.6	1 cc	1 cc	1 cc		0	
23	4	1 cc	1 cc	1 cc		7,000	
24	5	1 cc	1 cc	1 cc		210,000	
25	6	1 cc	1 cc	1 cc		200,000	
26	7	1 cc	1 cc	1 cc		230,000	
27	8	1 cc	1 cc	1 cc		200,000	
28	9	1 cc	1 cc	1 cc		240,000	

Result — The limit of the bactericidal activity lies for all pH steps at 1/100000 (or according to the final dilution of the anacardate at 1/300000); the stronger pH concentrations 3,6 and 4, cannot be taken into account, as they themselves kill the exposed germs (cf. control tube 22 and 23). At a 1/3000000 concentration the anacardate is inactive at all pH steps (*).

The lack of influence of the pH concentration on the bactericidal activity of anacardic acid places this substance apart from other anionic detergents, with whom it otherwise shows great similarity of action.

As we have pointed out elsewhere (Eichbaum, Hauptmann and Rothschild) this unusual behaviour of anacardic acid might be explained by the fact that the hydrolytic dissociation of anacardic acid seems to follow the scheme



This means that even in (weakly) alkaline solutions, undissociated anacardic acid is present, which is responsible for the bactericidal action. According to Osterhout, the undissociated molecules possess a greater ability to enter the cell and are therefore more active than the ionized compounds.

Inhibiting factors

Both serum and lecithin have an inhibiting effect on the bactericidal activity of Na anac, as bas been observed also with other anionic detergents. The germicidal power of Na anac and related products, in the presence of approximately 25% of serum, is reduced to 1/100 — 1/500 part of the activity in saline medium. The formation of insoluble Calcium salts cannot be responsible for this phenomenon, since the addition even of an excess of Sodium citrate does nos modify the result.

The inhibiting effect of two commercial egg lecithin preparations ("P and E") differed considerably, although both samples were marked as "pure".

0.5 cc of a 1/1000 dilution of lecithin "E" neutralized completely the staphylococcidal activity of 0.5 cc of a 1/10000 Na anac. solution, whereas the inhibition caused by lecithin "P" under the same condition was only partial (**).

(*) The test with anthrax bacilli gave in general the same result as the test with staphylococci.

(**) The lecithin emulsions were prepared from a 1% stock sol. in alcohol, by adding physiol. saline solution. A control test with the same amount of alcohol as contained in the 1/1000 lecithin-emulsion did not show any interference with the bactericidal activity of Na anac.

An analogous result was obtained, using instead of egg lecithin, autolysates of gram-positive germs (Staphylococci) or gram-negative bacteria (coli bacilli).

The inhibiting action of lecithin can also be easily demonstrated by the hanging drop method. Motile gram-positive bacilli, suspended in lecithin + an otherwise imobilizing concentration of Na anac, remain motile for many hours.

Pus, from an acute phlegmone, containing numerous leucocytes and staphylococci, did not diminish appreciably the sterilizing power of a 1/200 solution of Na anac.

Relation between chemical structure and antibacterial activity

(cf. Table No. V) (*)

Various structural factors seem to influence the bactericidal activity of anacardic acid.

Salicylic acid, which possesses the same aromatic nucleus, with the carboxyl and the hydroxyl groups in identical positions as anacardic acid, but no alkyl radical, is devoid of the specific activity of anacardic acid. Linoleic and ricinoleic acid, that are similar to anacardic acid in the structure of the side chain, but which do not contain the aromatic nucleus, display a similar activity, though to a definitely lesser degree. *It can be concluded therefrom that coexistence of an aromatic nucleus and a long aliphatic side-chain is indispensable for the high bactericidal activity of anacardic acid.*

The saturation of the side-chain does not seem to be of special significance, since the tetrahydro-anarcadic acid and its sodium salt possess only a slightly lower activity than the unsaturated compounds.

There exists, however, one striking difference between the saturated and the unsaturated compounds, which we are at a loss to explain satisfactorily. Whereas anacardic acid and sodium anacardate display full activity in liquid as well as in solid media, the saturated products act only in liquid media. Even crystalline sodium tetrahydro-anacardate, when placed in the centre of an inoculated agar dish, does not prevent the growth of staphylococci up to the very border of the substance. This cannot be due only to a low diffusion of this substance: Crystalline Na tetrahydro-anacardate was placed in the centre of a sterile agar dish and allowed to diffuse into the surroundig gel during 24 hours at 37°; only after this interval the whole agar dish was inoculated with a suspension of staphylococci. Even then, not the slightest inhibition of bacterial growth could be noticed. In striking contrast to this observation is the fact that this subs-

(*) Cf. F. Eichbaum, H. Hauptmann, H. Rothschild, l.c.

tance, in watery solution, has about the same high bactericidal titre as the unsaturated analogon.

In order to analyse the special importance of the free hydroxyl group in the aromatic nucleus, we tested the derivatives, in which the hydrogen of these groups was substituted.

Three types of compounds are possible: one, in which the hydrogen of the phenolic hydroxyl was substituted (methylether of anacardiac acid = 2 metoxy — 6 pentadecadienyl benzoic acid); a second, in which the hydrogen of the carboxyl group was substituted (ethyl anacardate); a third, in which both hydroxyls were substituted (2 metoxy — 2 — pentadecadienyl methylbenzoate).

The comparison of the bactericidal activity of the various substances enumerated in Table No. V shows that the etherification of the phenolic hydroxyl-group has little influence on the activity, since the sodium salt of the methylether owns a bactericidal power only slightly inferior to anacardic acid. Neither does the esterification of the carboxylgroup lead to a complete extinction of the germicidal activity. On the other hand, the simultaneous substitution of both hydrogens renders the compounds almost inactive. Corresponding results have been obtained with tetrahydro-anacardic acid, although the methylation of the phenolic hydroxyl group seems to diminish much more the activity of this substance than is the case with the non-hydrogenated acid.

The inactivation of anacardic acid by introduction of two methyl groups into the phenol and carboxyl group, respectively, is not restricted to the bactericidal activity alone. As will be shown later, anacardic acid inhibits also the plasma coagulating activity of certain snake venoms; the same holds true for tetrahydroanacardic acid, whereas the dimethyl-products are devoid of any toxin destroying activity.

All these products show, apart from quantitative differences, the same specificity of their antibacterial power, which s directed principally against gram positive germs; in all instances, the bactericidal activity was reduced in the presence of either serum or lecithin.

Three more products, not included in table N° V should be mentioned here.

1. An addition-product of sodium anacardate and sulfanilamide, which showed exclusively the characteristics of the anionic detergent and whose action in-vitro, was not inhibited by p-amino-benzoic acid. The product did not reveal any chemotherapeutic activity against a virulent strain of hemolytic streptococci (*).

(*) Experiments on mice.

2. Copperanacardate (*). The recently prepared substance is highly soluble in lipoid solvents (ether, acetone, petrolether, chloroform). The aged salts, even a few hours after the preparation, become absolutely insoluble in either water or lipoid solvents. An emulsion prepared from the acetone solution showed a high bactericidal power against both gram-positive and gram-negative germs, the latter activity being probably due to the dissociation of oligodynamically active copper ions. The bactericidal power against the gram-positive germs was stronger than against the gram-negative ones; this shows that the antibacterial activity is not due only to the presence of Copper ions, which act mainly on the gram-negative bacteria (Neisser and Eichbaum). The growth of molds was not inhibited by Copperanacardate.

3. Silveranacardate, (*) which is soluble only as a complex in ammonia, shows, like the coppersalt, a high activity against both gram-postive and gram-negative bacteria. In part, this activity might be due to the high alkalinity of the substance.

TABLE No. V

(pag. 89/90)

Bactericidal action of anacardic acid and related compounds (**) against Staphylococcus haemolyticus aureus.

Technique — Each substance (No. 1-20) was tested in 5 progressive dilutions (1:100—1:1,000,000) which were made up with physiologic saline. 1 cc of each dilution remained in contact with 1 cc of a staphylococcal suspension, for 15' at room temperature.

Then 0.1 cc was withdrawn from each tube and tranferred to a Petri dish where it was mixed immediately with liquefied agar (40°). The solidified agar dishes were placed into the incubator at 37°; after 48 hours the grown colonies were counted and the results were registered, as follows:

O sterility
\+ weak growth
++ medium growth
+++ full growth (equal to the control)

Each series of tested substances included one control tube, which contained 1 cc of saline solution + 1 cc of staphylococcal suspension.

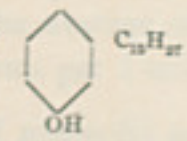
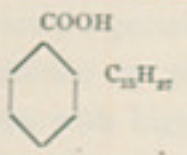
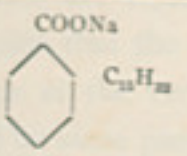
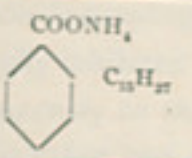
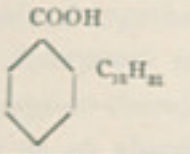
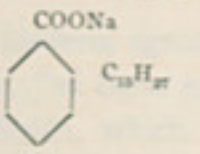
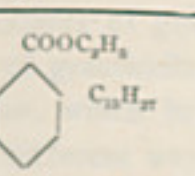
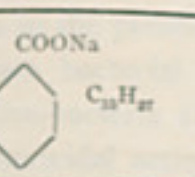
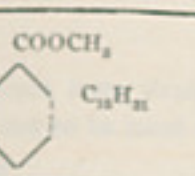
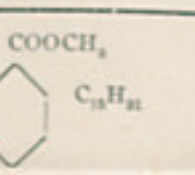
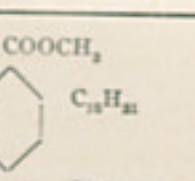
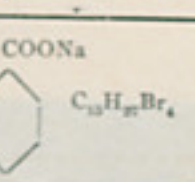
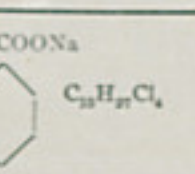
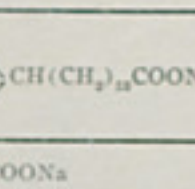
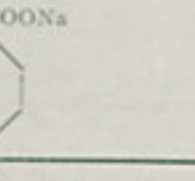
(*) Details on the interesting physico-chemical properties of these substances will be described elsewhere by H. Hauptmann and H. Rothschild.

(**) The "Cardol" mentioned in the following table was obtainable only in an impure form and contained certainly still a high percentage of anacardol.

Bactericidal action of anacardic acid and related compounds against Staphylococcus haemolyticus aureus.

TABLE No. V

DILUTIONS

No.	Substance	Formula	$5x10^{-3}$	$5x10^{-4}$	$5x10^{-5}$	$5x10^{-6}$	$5x10^{-7}$	Control in saline	Type of solution
1	Crude Cashew oil	—	0	0	0	+-++	+++	+++	emulsion in alcohol-water
2	Cardol		0	0	+	++	+++	+++	idem
3	Anacardol		0	0	0	+	+++	+++	idem or emulsion with gum arabic
4	Anacardic acid		0	0	0	+	++-+++	+++	like No. 3
5	Sodium anacardate		0	0	0	0-+	++-+++	+++	solution in water
6	Ammonium anacardate		0	0	0	0-+	++-++	+++	idem
7	Tetrahydro-anacardic acid		0	0	0-+	+-++	+++	+++	emulsion with gum arabic
8	Sodium tetrahydro-anacardate		0	0	0	+	+++	+++	solution in water
9	Ethylanacardate (Ethylester of anacardic acid)		0	0	+	+++	+++	+++	emulsion with gum arabic
10	Sodium methyl-anacardate (Methylether of Na anacard.)		0	0	0	+	++-+++	+++	solution in water
11	Sodium methyl-tetrahydro-anadate (Dimethylanacardic acid)		+	++	+++	+++	+++	+++	emulsion with gum arabic
12	Sodium methyl tetrahydro-anacardate (Methylether of Na tetrahydro anac.)		+	++	++-+++	+++	+++	+++	idem
13	Methylether of methyltetrahydro-anacardate (Dimethyltetrahydro-anacardate)		++-+++	++-+++	+++	+++	+++	+++	idem
14	Sodium tetrabrom-anacardate		0	0	+	++	+++	+++	idem
15	Sodium tetrachlor-anacardate		0	0	0	++	+++	+++	emulsion with gum arabic
16	Sodium linoleate	$C_{17}H_{31}COONa$	0	+-++	++-+++	+++	+++	+++	solution in water
17	Sodium ricinoleate	$C_{17}H_{32}OCOONa$	0	+	+++	+++	+++	+++	idem
18	Sodium oleate	$C_{17}H_{33}OCOONa$	++	++-+++	+++	+++	+++	+++	idem
19	Sodium chaulmoograte		++-+++	+++	+++	+++	+++	+++	idem
20	Sodium salicylate		+++	+++	+++	+++	+++	+++	idem

Readings: 0 no growth; + weak growth; ++ medium growth; +++ full growth.

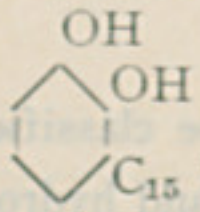
The test strain, used in these experiments, was an hemolytic coagulase-positive staphylococcus (aureus) which had been isolated recently from an abscess (Strain G 7 of our collection). The average number of staphylococci in the "suspension" amounted to 200000 germs per 1 cc).

Discussion — The alkali salts of anacardic acid can be classified as anionic detergents since they possess the characteristic hydrophobe and hydrophile groups, the active part being located in the anionic complex. Accordingly, they show the characteristics common to all anionic detergents (**) : high surface activity, emulsifying power, bactericidal activity directed especiallà against gram-positive germs, toxin-destroying activity, specific inhibition by phospholipids. It is interesting to note that other surface-active agents with high bactericidal activity like gramicidin and certain toxic wheat proteins are equally inactivated in the presence of phospholipids (Dubos). Dubos holds that the phospholipids exert a "protective action on the cell surface, perhaps at the very sites where the toxic agent would otherwise become absorbed." The same phenomenon takes place, however, in a cell free medium as well: the toxin-destroying action of anacardic acid, ricinoleate and other detergents is specifically inhibited by lecithin, and *this only when toxin and lecithin are exposed simultaneously to the action of the detergent, or if lecithin and the detergent are mixed together and the toxin is added afterwards. If, however, toxin and detergent are mixed together and lecithin is added shortly afterwards, the atoxic complex (toxin-detergent) is not dissociated* (***). This speaks, in our opinion, in favor of a physico-chemical union between lecithin and the detergent, without the interference of an absorbing cellreceptor.

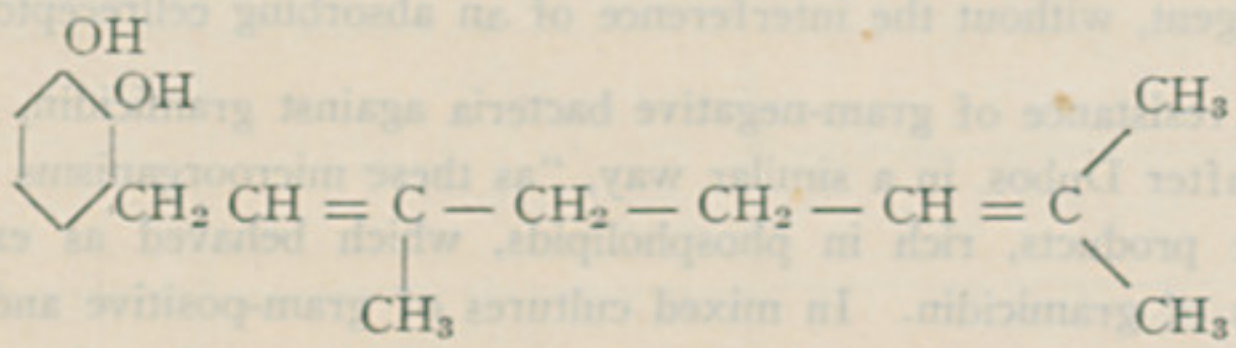
The resistance of gram-negative bacteria against gramicidin, etc. can be explained. after Dubos. in a similar way, "as these microorganisms produce certain metabolic products, rich in phospholipids, which behaved as extremely active inhibitors of gramicidin. In mixed cultures of gram-positive and gram-negative bacilli, the latter suppressed the action of gramicidin on the otherwise very sensitive microorganisms." The anacardates, which have chemically no relation to the polypeptide gramicidin, behave in a different way, since their activity on gram-positive germs remains unimpaired even in the presence of great amounts of gram-negative bacteria. Autolysates of dense bacterial suspensions of either gram-positive (staphyiococci) or gram negative-bacteria (B. coli) are of equal potency as to their inhibiting power on the bactericidal action of the anacardates.

(**) Among the anionic detergents derived from straight chain alkyl compounds, those with 12-16 C atoms in the aliphatic chain proved to be most active (Baker, Harrison, Miller).

(***) Further details cf. the next paper.

Numerous plants, belonging to various families, produce substances with a structure similar to anacardic acid and cardol. From a medical point of view, the most important among them is urushiol ("poison ivy" from Rhus toxicodendron)  $C_{15}\ H_{27}$, the substance responsible for many cases of toxic dermatitis. A similar skin-irritating effect has been observed with Bhilawanol (from Semicarpus anacardium), which is chemically identical with urushiol (Goldsmith). To our knowledge, bactericidal and other biological tests with Urushiol, Bhilawanol, etc., have not been performed so far, though the resemblance of these substances to anacardic acid and cardol, would suggest a similar biological activity. Keil and others found in persons sensitive to poison ivy a cross-reaction with cashew (raw) oil, cardol, anacardic acid, anacardol and cardanol; the incidence and intensity of group reactions were definitely less with the hydrogenated products, such as tetrahydrocardol, tetrahydro-anacardic acid and tetrahydro-anacardol. Hexylresorcinol gave no group reaction. In our own experiments on normal individuals, we could not observe any dermatitis-producing effect, even after a repeated treatment with 1 — 2% ointments of sodium anacardate or cashew nut oil. (*).

Among other substances of similar chemical structure two more should be enumerated here; pelandjauic acid $C_{17}H_{31}C_6H_3OHCO_2H$ (from the tree Pentaspadon Motleyi Hook) which possesses 2 Carbon atoms more than anacardic acid, and 3 geranyl catechol



All these substances have been known for their skin irritating activity, which seems in part due to the OH groups in the ring (Sizer and Prokosch).

Surface active substances with bactericidal activity have also been isolated from lower plants such as certain fractions of pyocyanase (Pseudomonas aeruginosa: Birch-Hirschfeld), and gramicidin and tyrocidin from bac. brevis. According to Herrell, gramicidin "appeared to behave somewhat as an anionic detergent" which does not prevent its destruction by other anionic or cationic

(*) Raw cashew nutshell liquid, when left in contact with the skin surface during 1 hour, produces a servere dermatitis after a 24 hour's interval.

detergents. It has in common with other anionic detergents the specific action against gram positive bacteria and the inactivation by serum and phospholipids. On the other hand, tyrocidin acts more like a cationic detergent. "The mode of action of gramicidin and tyrocidin may differ in degree or kind and may involve different combinations of interference with normal cellular metabolism, however there seems little doubt that both substances behave biologically in a manner similar to that of certain detergents" (Herrell).

ABSTRACT

The sodium salt of anacardic acid, one of the main constituents of Cashew nut oil, belongs to the most active anionic detergents. Apart from its high staphylococcidal power, equalled by few members of this group, it is also very active against other gram-positive bacteria up to dilutions varying from 1/200000 — 1/2000000, at a contact time of 15'. Amongst the gram-negative germs gonococci and meningococci are highly sensitive to Na anacardate; to a lesser degree also Brucellae, Pasteurellae and Influenza bacilli. The only sensitive germs amonst the Enterobacteriaceae are the proteus bacilli which, being more resistant than gram-positive bacteria, are killed only by higher concentrations (1/200 — 1/2000), whereas weaker concentrations suppress only the growth of the H forms. Tubercle bacilli are less sensitive than other gram-positive bacteria, but they die also after a 30 minutes' contact with a 1/100000 solution (human strain Ratti; a bovine strain proved to be somewhat more resistant). The Spirochaeta gallinarum is highly sensitive. Yeasts and molds, with few exceptions, are very resistant. The vegetative forms of anaerobic bacilli are readily killed; the spores of Cl. septicum and Cl. tetani survive even a prolonged contact (1 hour) with a 1/100 solution, whereas the spores of Cl. perfringens and of aerobic bacilli (anthrax) are rather sensitive. Proteins (serum) reduce the bactericidal activity of Na anacardate, pus only slightly. Similarly, lecithin and concentrated autolysates from gram-positive as well as gram-negative bacteria lower the bactericidal power of Na anacardate. Experiments on animals (mice) did not reveal any chemotherapeutic activity of sodium anacardate. The bactericidal activity of Na anacardate is largely independent of the actual pH, which might be explained by the probable presence of undissociated anacardic acid even in (weakly) alkaline solutions.

18 subproducts of anacardic acid and Cardol were studied and compared as to their bactericidal power. It could be shown that neither the saturation of the side chain, nor the etherification of the phenolic group, nor the esterification of the carboxylic group, abolish the germicidal activity of anacardic acid. This can be achieved only by simultaneous substitution of both hydrogens at the ring.

The high bactericidal activity of anacardic acid seems related to the coexistence of an aromatic nucleus and a long aliphatic side chain. The relation between anacardic acid, cardol and similar substances of vegetal origin (Urushiol, Bhilawanol, Pelandjauic acid etc.) is discussed.

RESUMO

O sal de sódio do ácido anacárdico, um dos constituintes principais do oleo de cajú, pertence ao grupo dos detergentes aniônicos mais ativos. Além de seu alto poder antiestafilocócico, igualado por poucos membros dêste grupo, êle é também ativo contra outras bactérias gram-positivas, com um tempo de 15 minutos de contacto, até diluições que variem de 1/200000 a 1/2000000. Entre os germes gram-negativos, os gonococos e meningococos são altamente sensíveis ao anacardato de sódio e, em menor gráu, também as Brucelas, Pasteurelas e bacilos da influenza. Entre as Enterobactériaceas os únicos sensíveis são os bacilos Proteus; êles são mais resistentes do que as bactérias gram-positivas e só morrem em concentrações mais altas de anacardato sódico (1/200 a 1/2000) enquanto que concentrações mais fracas somente suprimem o crescimento das formas H. Os bacilos da tuberculose são menos sensíveis do que outras bactérias gram-positivas, mas morrem também depois de 30 minutos em contacto com uma solução a 1/100000 (raça Ratti humana; uma raça bovina mostrou-se um pouco mais resistente). O Spirochaeta gallinarum é altamente sensível. Leveduras e bolores, com poucas exceções, são muito resistentes. As formas vegetativas dos bacilos anaeróbios morrem prontamente; os esporos de Cl. septicum e Cl. tetani sobrevivem a um contacto prolongado (1 hora) com uma solução a 1/100, enquanto que os esporos de Cl. perfringens e de bacilos aeróbios (antrax) são mais sensíveis. As proteinas (do sôro) reduzem a atividade bactericida do anacardato de sódio e o pus só reduz fracamente. Do mesmo modo, lecitina e autolizados concentrados, tanto de bactérias gram-negativas como positivas, abaixam a atividade bactericida do anacardato de sódio.

As experiências em animais (camundongos) não revelaram qualquer atividade quimioterápica do anacardato de sódio. Sua atividade bactericida é largamente independente do pH o que pode ser explicado pela provável presença de ácido anacárdico não dissociado mesmo em soluções (fracamente) alcalinas.

Foram estudados 18 subprodutos do ácido anacárdico e do Cardol, bem como comparadas suas atividades bactericidas. Tanto a saturação da cadeia

lateral, como a eterificação do grupo fenólico ou a esterificação do grupo carboxílico, não puderam abolir a atividade germicida do ácido anacárdico. Isto só foi conseguido pela substituição simultânea de ambos os átomos de hidrogênio do núcleo.

A alta atividade bactericida do ácido anacárdico parece estar ligada à coexistência na molécula de um núcleo aromático e de uma longa cadeia lateral alifática. A relação entre ácido anacárdico, cardol e substâncias similares de origem vegetal (Urushiol, Bhilawanol, ácido Pelandjauico, etc.) é discutida.

Acknowledgements — I wish to express my gratitude to prof. H. Hauptmann and to Miss H. Rothschild of the Chemical Department of the Faculdade de Filosofia, Ciencias e Letras, São Paulo, for the laborious preparation of most of the mentioned products and for many valuable suggestions that have stimulated this work. I am equally indebted to Dr. H. Gleich and Miss B. Schwarzaid for the preparation of another part of the enumerated compounds. I also wish to thank Prof. R. Wasicky of the Faculdade de Farmacia e Odontologia, São Paulo, for the encouraging interest he has taken in this study.

REFERENCES

1. *Anonymous.* Penicillinlike substances from higher plants (Editorial), *J. Amer. Med. Assn.*, **127**(9) :522-523, 1945.
2. *Baker, Z.; Harrison, R. W. & Miller, B. F.* Action of synthetic detergents on the metabolism of bacteria, *J. Exper. Med.*, **73** :249-271, 1941.
3. *Birsch-Hirschfeld, L.* Versuche zur Analyse der Pyocyanase, *Zschr. f. Hyg. u. Inf. Kr.*, **116** :304-314, 1934/35.
4. *Dubos, R. J.* Antimicrobiological agents of biologic origin, *J. Amer. Med. Assn.*, **124**(10) :633-637, 1944.
5. *Eichbaum, F. W.; Hauptmann, H. & Rothschild, H.* Preparação e ação biológica de ácido anacárdico e alguns derivados, *Anais Assoc. Quim. Brasil.*, 4 :83-94, 1945.
6. *Furtado, J. J.* Sobre o oleo de Cajú, *Rev. Brasil. de Farm.*, **26**(2) :7-10, 1945.
7. *Goldschmith, N. R.* Dermatitis from Semicarpium anacardium (Bhilawanol or the marking nut) spread by mail, *J. Amer. Med. Assn.*, **123**(1) :27, 1943.
8. *Hansen, G. A. & Looft, C.* Leprosy in its clinical and pathological aspects, Chapter VIII "Treatment", Bristol, 1895.
9. *Herrell, W. E.* Penicillin and other antibiotic agents, Philadelphia and London, W. B. Saunders Co., 1945.
10. *Keil, H.; Wasserman, D. & Dawson, C. H.* The relation of hypersensitiveness to poison ivy and cashew nutshell liquid, *Science*, **102**(2646) :279-280, 1945.
11. *Keil, H.; Wasserman, D. & Dawson, C. H.* The relation of chemical structure in catechol compounds and derivatives to poison ivy, hypersensitiveness in man as shown by the patch test, *J. Exper. Med.*, **80**(3) :275-287, 1944.
12. *Lacerda, P. Araujo Feio.* Anacardium occidentale L. Cajueiro, *Rev. Flora Med.*, **8**(1/6) :39-87, 1941.

13. *Larson, W. P.* The influence of the surface tension of the culture medium on bacterial growth, *Proc. Soc. Exper. Biol. a. Med.*, **19**:62-63, 1921/22.

14. *Larson, W. P.; Evans, R. D. & Nelson, E.* The effect of sodium ricinoleate upon bacterial toxins and the value of soap toxin mixtures as antigens, *Ibid.*, **22**:194-196, 1924/25.

15. *Larson, W. P. & Montank, I. A.* The effect of wetting on the pathogenicity and viability of the tubercle bacillus, *Ibid.*, **20**:229-232, 1922/23.

16. *Larson, W. P. & Nelson, E.* The effect on the surface tension of the medium upon bacterial growth, *Ibid.*, **21**:278, 1923/24.

17. *Lima, A.* Tratamento da Lepra, *Brasil Medico*, **9**(4):25-28, 1895.

18. *Lominsky, I. & Lendrum, A. C.* The effect of surface active agents on Bac. proteus, *J. Path. a. Bact.*, **54**:421-433, 1942.

19. *Lucas, E. H. & Lewis, R. W.* Antibacterial substances in organs from higher plants, *Science*, **100**(2609):597-599, 1944.

20. *Morais, M. O.* O oleo de cajú na lepra, *Rev. Brasil. de Farm.*, **26**:17-20, 1945.

21. *Neisser, M. & Eichbaum, F. W.* Die oligodynamische Metallwirkung in Theorie und Praxis, *Erg. Hyg., Bakt. Immunitätsf.*, **13**:170-226, 1932.

22. *Osborn, E. M.* On occurrence of antibacterial substances in green plants, *Brit. J. Exper. Pathol.*, **24**:227-231, 1943.

23. *Osterhout, W. J. V.* Quoted by Baker and coll. (2).

24. *Sizer, I. W. & Prokesch, C. E.* Inactivation of the irritant toxicants of poison ivy and related compounds by tyrosinase, *Science*, **101**:517-518, 1945.

25. *Staedeler* (quoted by:) *Jahresber. d. Chemie*, **1**:574, 1847/48.

N.B. For the technical literature on cashew nut oil and subproducts cf. Damitis, F. M. "Cashew Nut Oil" in "Protective and Decorative Coatings", Vol. I, New York, John Wiley and Sons, Inc., p. 74-101, 1941.