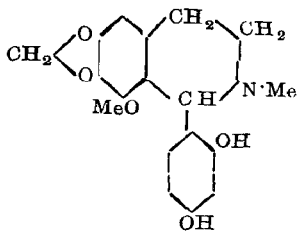
Studies in Chemotherapy. Part III. (Attempts to Prepare Antimalarials). Derivatives of Cotarnine.

By Gurcharan Singh Ahluwalia, Basheshar Das Kochhar and Jñanendra Nath Rây.

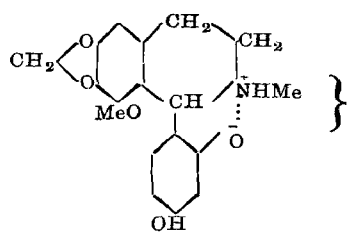
In the report of Opium Commission of 1893 (Government of India) it is stated that opium may have an antimalarial action. Narcotine is stated to resemble quinine in its tonic and anti-periodic properties. Gordon (*Indian Annals of Medical Science*, Vol. VII) confirmed the prophylactic value of narcotine. But recently Chopra and Knowles (*Ind. J. Med. Res.*, 1930, **18**, 5) have arrived at the conclusion that narcotine has no curative or prophylactic value in malaria, even in large doses. At the suggestion of Central Board of Revenue (Government of India) the present investigation of converting narcotine into a febrifuge, preferably an antimalarial, was undertaken.

Cotarnine has now been condensed with phenols, *e.g.*, phloroglucinol, resorcinol and pyrogallol, the reaction proceeding with diminishing ease in the order given. It has been established (Ahluwalia, Narang and Rây, *J. Chem. Soc.*, 1931, p. 2057) that in these condensations probably the nuclear hydrogen atom of the phenol molecule takes part. Anhydrocotarnine-resorcinol (I) or (IA) * has now been found to have antipyretic action favourably comparable to quinine and its toxic action to *paramoecium* (*cf.* its structure with hexyl resorcinol) is greater in equivalent dilution than quinine. The details of these experiments will be published elsewhere. Cotarnine also condenses with 1-phenyl-3-methylpyrazolone, 3-methylpyrazolone, 3:5-dimethylpyrazole, 3-methyl-5-phenylpyrazole, 1:5-diphenyl-3-methylpyrazole to give corresponding anhydrocotarninopyrazoles. In the formation of these substances (III) probably a carbon to carbon linkage is established since 1:5-diphenyl-3-methylpyrazole (II) can only react if such is the case.

* Certain results obtained since the paper was written up indicate that the substance may also be (IA).



(I)



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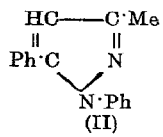
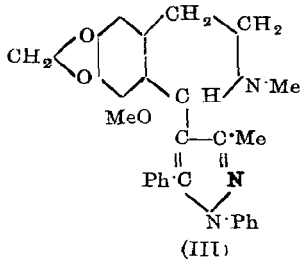


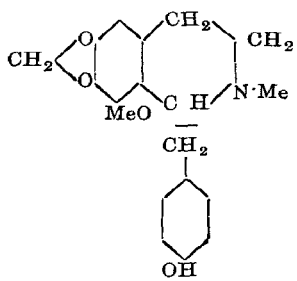
(II)



(III)

Foulds and Robinson (*J. Chem. Soc.*, 1914, **105**, 1970) also assume that in the condensation of cotarnine with α-methylindole, neither the imino group nor the methyl group was involved but the condensation took place at the β-carbon atom of the indole nucleus.

Hope and Robinson's *anhydrocotarnino*-p-*nitrotoluene* (*J. Chem. Soc.*, 1911, **99**, 2114) has now been reduced to the corresponding amino compound and from it a new alkaloid (IV), which may be regarded as belonging to laudanosine group has been prepared.



(IV)

Certain of the above compounds have been tested in respect of their antimalarial action. The numbers given in parentheses in the experimental portion, are to facilitate reference to biological tests. The antipyretic properties of the compounds described in the paper has been investigated. The results are given at the end of the paper. The antimalarial properties are being studied.

Experimental.

Oxidation of Narcotine to Cotarnine.

The following process worked better than the method given by Rakshit (*J. Chem. Soc.*, 1918, **113**, 469).

Nitric acid (*d* 1·4,66 *g.*) in water (160 c.c.) was heated till the temperature was 49°. To this finely powdered narcotine (20 g.) in small portions was added with vigorous shaking, the whole operation lasting 1½ hours, the temperature being maintained at 49° all the time. The felt-jacketed reaction vessel, which may also be a thermo-flask, was set aside for 12 hours, and then the solution filtered from the oily deposit. The strongly cooled filtrate was basified with concentrated sodium hydroxide solution, the precipitated cotarnine collected, washed with ice-water, dried and crystallised from benzene, m.p. 132°, yield 10·5 g. The mother liquor furnished opianic acid on acidification.

Anhydrocotarnino-resorcinol (I): 1-(2′:4′-*Dihydroxyphenyl*) *hydrocotarnine* (R 76).

A mixture of resorcinol (2·2 g.), cotarnine (4·7 g.) and alcohol (*absolute*, 52 c.c.) was warmed on the steam bath at 40-45° for 15 minutes. The pale yellow crystalline deposit well washed with alcohol, was collected after standing some time, yield 6 g. (*cf.* Liebermann, *Ber.*, 1904, **37**, 2744). The substance does not dissolve in benzene and ether but dissolves readily in dilute acid and alkali solutions without hydrolysis. (Found: N, 4·3. $C_{18}H_{19}O_5N$ requires N, 4·3 per cent.).

The substance (2 g.) was mixed with *N*/2-HCl solution (12·2 c.c.) and the clear solution cooled when the *hydrochloride* (R 77) crystallised out in short prisms, m.p. 240°. (Found: N, 4·1. $C_{18}H_{19}O_5N$, HCl requires N, 3·8 per cent.).

The substance in 1:15,000 dilution completely arrests the movement of *paramoecium* and kills 20 per cent. in 54 hours

whilst quinine is without effect at the same concentration on the same strain of *paramoecium*. The substance decreases the tone and amplitude of the contractions of muscles and produces an immediate contraction in isolated uterus and thus differs from cotarnine which causes slow tonic contraction. The uterus does not relax after washing with Ringer's solution, as is the case with adrenaline; thus it resembles quinine hydrochloride. The substance has no effect on the shape of contraction of striped muscle. In a dilution of 1:10,000 to 1:50,000 it causes diostalic dilatation with decreased amplitude of contraction of the cardiac muscle; the frequency is also decreased which is followed later on by impared conductio ı of impulses resulting in partial or complete heart block. However, the vagus endings are not involved in the effect of the drug on heart, therefore, it directly acts on the cardiac muscles.

1-(2:3:4 *Trihydroxyphenyl*) *hydrocotarnine* (R 91) similarly prepared from pyrogallol (2·5 g.) and cotarnine (4·7 g.) in alcohol (15 c.c.) had m.p. 211°. (Found N, 4·2. $C_{18}H_{19}O_6N$ requires N, 4·1 per cent.).

1-(2:4:6- *Trihydroxyphenyl*) *hydrocotarnine* (R 87), m.p. 170° and the *hydrochloride*, m.p. 185° (decomp.) were similarly prepared. (Found: N, 3·4. $C_{18}H_{19}O_6N$, HCl requires N, 3·7 per cent.).

Anhydrocotarnino-1-phenyl-3-methylpyrazolone (R 88) (formula analogous to III.)—A mixture of 1-phenyl-3-methylpyrazolone (3·5 g.) in absolute alcohol (20 c.c. containing sodium ethylate from 0·2 g. of sodium) and cotarnine (4·7 g.) was heated on the steam-bath till a clear solution resulted, then on standing a colourless crystalline material deposited, m.p. 177°, yield 6·7 g. (R 73).

The substance is insoluble in benzene, acetone and chloroform but is soluble in hot alcohol and with decomposition in hot water. At 155°, the substance becomes brownish yellow. (Found: N, 10·5. $C_{22}H_{23}O_4N_3$ requires N, 10·7 per cent.).

Similarly, when a solution of 3-methylpyrazolone (2·0 g.) in hot alcohol (10 c.c.) was added to a hot solution of cotarinine (4·7 g.) in alcohol (15 c.c.), the corresponding anhydrocotarninopyrazolone, m.p. 199° (decomp.) crystallised out on standing. The substance is phototropic. It dissolves to a colourless solution in hydrochloric acid without apparent decomposition. (Found: N, 13·5. $C_{16}H_{19}O_4N_3$ requires N, 13·3 per cent.).

3:5-*Dimethylpyrazole* (3 g.), prepared from acetylacetone and hydrazine hydrate, when interacted with a hot solution of cotarnine (4 g.) in alcohol furnished *anhydrocotarnino-3:5-dimethylpyrazole*

after standing 4 hours, m.p. 140° after recrystallisation from hot alcohol. (Found: N, 13·6. $C_{17}H_{21}O_3N_3$ requires N, 13·3 per cent.). *Anhydrocotarnino-3-methyl-5-phenylpyrozole*, m. p. 146° after changing colour at 137° (R 95) was similarly prepared from the corresponding pyrazole. (Found: N, 11·4. $C_{22}H_{23}O_3N_3$ requires N, 11·2 per cent.).

Anhydrocotarnino-1:5-diphenyl-3-methylpyrazole (R 98) was deposited from a warm solution of cotarnine (5 g.) and 1:5-diphenyl-3-methylpyrazole (5 g.) in absolute alcohol (15 c.c.) after standing for 1 hour and had m. p. 148° after crystallisation from alcohol. (Found: N, 9·6. $C_{28}H_{27}O_3N_3$ requires N, 9·3 per cent.).

Anhydrocotarnino-p-nitrotoluene can be obtained in a good yield by the following modification of Hope and Robinson's process (*loc. cit.*). Cotarnine (10 g.), and *p*-nitrotoluene (15 g.) in dry alcohol (100 c.c. containing sodium ethylate from 1 g. of sodium) was warmed at 40-45° for 15-20 minutes. The product obtained after standing 12 hours had m.p. 121°, yield 8 g. No difficulty was experienced in reducing the substance (*cf.* however, Gulland and Virden, *J. Chem. Soc.*, 1929, p. 1793). The nitro compound (9 g.) was gradually added to a mixture of stannous chloride (18 g.) hydrochloric acid (*d* 1·16, 36 c.c.) water (30 c.c.) and was shaken for 20 hours at 28-32°. The clear filtrate obtained after dilution with water furnished the amino compound when strongly basified with sodium hydroxide solution. The substance separates as pale yellow needles, m. p. 95° from hot dilute alcohol, yield 6·8 g. (Found: N, 8·6. $C_{19}H_{22}O_3N_2$ requires N, 8·6 per cent.).

Anhydrocotarnino-p-hydroxytoluene (IV, R 90).—A solution of anhydrocotarnino-*p*-aminotoluene (3·5 g.) in sulphuric acid (20 c.c. of 10 p. c.) was diazotised with a solution of sodium nitrite (0·7 g.) in water (3 c.c.) at 0°. The mixture was allowed to stand at room temperature for a few days, then made alkaline with sodium carbonate, and the precipitated phenol thrice crystallised from hot dilute alcohol (charcoal), m. p. 191°. (Found: N, 4·5. $C_{19}H_{21}O_4N$ requires N, 4·3 per cent.).

Anhydrocotarnino-p-phenetidide (R 96) was obtained from cotarnine (4·7 g.), phenetidine (2·7 g.), alcohol (15 c.c.) and sodium (0·1 g.) by gentle warming for $\frac{1}{2}$ hour and then standing for 3-4 hours. The yield is much poorer without sodium ethylate. Recrystallised from a mixture of benzene and ligroin it melts at 126°, yield 4·2 g. (Found: N, 7·9. $C_{20}H_{24}O_4N_2$ requires N, 7·9 per cent.).

The *hydrochloride*, m. p. 239° (after crystallisation from methanol) was obtained from the benzene solution of the base by hydrogen chloride at 0°. Similarly *anhydrocotarnino-o-phenetidide* (R 97) m. p. 126° was prepared from *o*-phenetidine in a 85 p. c. yield, care being taken not to prolong the heating beyond ½ hour. (Found: N, 8·1. Calc. N, 7·9 per cent.).

Anhydrocotarnino-p-anisidide, m. p. 124° (Found: N, 8·3. $C_{19}H_{22}O_4N_2$ requires N, 8·2 per cent.) and the *o-anisidide*, m. p. 134° (Found: N, 8·3 per cent.) were prepared from *p*- and *o*-anisidine respectively.

Pharmacological.

[With Dr. Khem Singh Graval, M.B., B.S., Ph.D.]

Table I.

Effect of Different Dilutions of the 2′:4′-Dihydroxyphenylcotarnine Hydrochloride and Quinine on *Paramoecium* in 24 hours.

Dilution.	2′:4′-Dihydroxyphenyl-hydrocotarnine hydrochloride.	Quinine hydrochloride.
1 :40,000	All dead	All dead
1 :50,000	do	do
1 :75,000	do	do
1 :100,000	do	do
1 :150,000	20% dead	All alive
1 :200,000	All alive	do

Table II

Effect of 2′:4′-Dihydroxyphenylcotarnine Hydrochloride and Quinine Hydrochloride in higher Concentrations on *Paramoecium*.

Concentration of the drug used.	Movements become sluggish. Time in minutes.		Death Time in minutes.	
	2′:4′-Dihydroxy-phenylcotarnine hydrochloride (R 77).	Quinine hydrochloride.	2′:4′—Dihydroxy-phenylcotarnine hydrochloride.	Quinine hydrochloride.
1 :500	At once	At once	At once	At once
1 :1000	do	0·33	1·3	0·6
1 :2000	0·5	1·0	2—2·5	1·3
1 :3000	1·0	1·5	4·0	2·0
1 :4000	2·0	3·0	5·6	3·5
1 :8000	5 0	6·0	22·0	10·0
1 :16000	6·7	15·0	50·0	28—33

TABLE III.

Daily Record of the Rectal Temperature of Rabbits in F°.

Rabbits marked.	Date..	8 A.M.	11 A.M.	2 P.M.	5 P.M.
A	March 6th 1931	102·1	102·2	102·0	—
	7th 1931	102·4	101·7	102·2	103·2
	8th 1931	101·8	101·4	102·8	102·4
	9th 1931	102·0	102·1	102·8	103·0
B	6th 1931	102·2	102·2	102·2	—
	7th 1931	102·0	101·4	101·9	102·3
	8th 1931	101·9	101·4	102·0	101·4
	9th 1931	101·2	101·7	101·0	102·2
C	6th 1931	102·0	101·8	102·0	—
	7th 1931	102·0	101·1	101·6	101·6
	8th 1931	101·3	101·4	102·7	102·2
	9th 1931	101·8	102·8	101·6	102·8
D	6th 1931	101·7	102·3	102·5	—
	7th 1931	102·7	102·1	102·3	102·7
	8th 1931	102·4	101·9	102·8	102·7
	9th 1931	102.0	102·9	102·6	103·0
E.	6th 1931	102·0	101·3	101·4	—
	7th 1931	101·4	101·1	101·4	101·1
	8th 1931	101·8	101·2	101·4	101·6
	9th 1931	101·5	101·2	101·0	101·6

TABLE IV.

Change in temperature after injecting 1·5 c.c. of bacillus *coli communis* emulsion subcutaneously in the left thigh at 9·30 A.M. and the drugs (100 mg.) injected subcutaneously in the right thigh at 2 P.M. Temperature is shown in F° and the weight in kilos.

Time.	A.	B.	C.	D.	E.
Weight ...	1·64	1·45	1·7	1·1	1·5
9·30 A.M.	102·2	102·2	102·5	102·6	101·6
10·30	102·4	102·6	102·5	102·9	101·6
11·00	102·6	103·8	103·2	103·2	101·8
11·30	103·8	105·0	103·7	104·6	103·0
12·00	104·6	105·2	104·5	104·8	104·0
12·30 P.M.	104·7	106·0	105·8	105·0	104·4
1·00	104·8	106·0	105·6	105·4	105·0
2·00	104·8	106·0	105·6	105·5	105·0

TABLE IV—(*contd.*).

	Aspirine.	Quininine hydrochloride.	2′:4′ Dihydroxyphenylcotarnine hydrochloride.	Cotarnine hydrochloride.	*p*-Ethoxyaminophenylcotarnine hydrochloride.
2·30 P.M.	104·0	104·6	104·6	105·2	104·2
3·00	103·3	103·5	104·3	104·8	103·8
3·30	103·0	102·8	103·4	104·7	104·0
4·00	103·0	103·0	103·6	104·8	104·1
4·30	103·0	103·1	103·6	104·8	104·2
5·00	103·0	104·1	103·7	105·3	104·3
5·30	102·8	104·2	104·2	105·4	104·0
6·00	103·0	104·0	104·4	105·4	103·9
6·30	103·0	104·0	104·0	105·5	104·0
7·00	103·0	104·2	104·2	105·7	103·9
7·30	103·0	104·4	104·2	105·2	103·8
8·00	103·0	104·7	104·3	105·0	103·8
8·30	102·1	104·8	104·2	105·0	103·8
9·00	102·1	104·6	104·1	104·9	103·8
10·00	102·0	104·0	103·8	103·6	103·8

TABLE V.

Effect of Injecting 2′:4′-Dihydroxyphenylcotarnine Hydrochloride in the Ventral Lymp Sac of the Frogs.

Weight of the frog.	Dose per Kg.	Dose per 20g. body weight.	Actual dose.	Result.
20 g.	0·10 g.	2 g.	0·0020 g.	No effect.
30	0·10	2	0·0030	do
40	0·10	2	0·0040	Respiration slowed.
40	0·10	2	0·0040	No effect.
30	0·15	3	0·0045	do
15	0·15	3	0·0023	do
30	0·15	3	0·0045	Respiration slowed.
25	0·15	3	0·0037	No effect.
25	0·20	4	0·0050	Recovered.
15	0·20	4	0·0024	do
27	0·20	4	0·0054	Recovered.
15	0·20	4	0·0030	do
40	0·25	5	0·0100	do
45	0·25	5	0·0105	do
30	0·25	5	0·0075	Dead in 20 hrs.
25	0·25	5	0·0065	Recovered.
20	0·25	5	0·0050	do
12	0·25	5	0·0030	Dead in 7 hrs.
55	0·35	7	0·0192	Dead in 5 ,,
25	0·35	7	0·0087	Recovered.
30	0·35	7	0·0105	Dead in 5 hrs.
35	0·35	7	0·0122	Dead in 6 hrs.

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