γ-(*p*-Cyclohexanone)-butyric Acid (VII).—A mixture of 3.5 g. of γ-(*p*-hydroxycyclohexyl)-butyric acid (0.019 mole), 10 cc. of acetic acid and 40 cc. of water was placed in a flask equipped with a mechanical stirrer, a dropping funnel and a thermometer which was immersed in the liquid and warmed to 60°. After the acid had dissolved, the solution was cooled to 35° and kept at that temperature for the rest of the reaction.

An oxidizing mixture of 1.9 g. (0.0065 mole) of potassium dichromate, 6 cc. of concentrated sulfuric acid and 14 cc. of water was added over a period of fifteen minutes to the stirred solution. The reaction mixture was stirred for an additional thirty minutes and then it was allowed to stand until all of the oxidizing agent had been consumed. This usually required eight to ten hours. The mixture was then diluted with 200 cc. of water, saturated with sodium chloride and was extracted with ether. Upon evaporation of the ether, a yellow sirup remained which on cooling and scratching crystallized. The product was recrystallized from a mixture of ether and ligroin, yield 1.05 g. (30%), m. p. 81–82°.

Anal. Calcd. for $C_{10}H_{16}O_3$: C, 65.19; H, 8.76. Found: C, 64.91; H, 8.74.

The semicarbazone melts at 183–184°.

Anal. Calcd. for $C_{11}H_{19}O_3N_3$: C, 54.75; H, 7.94. Found: C, 54.99; H, 7.82.

Summary

1. 2-Hydroxy-3-[3′-*cis*-(4-hydroxycyclohexyl)-propyl]-1,4-naphthoquinone has been prepared and has been shown to be different from the low melting metabolite of 2-hydroxy-3-(3′-cyclohexyl-propyl)-1,4-naphthoquinone isolated by Fieser and associates.

2. The hydrogenation of γ-(*p*-cyclohexanone)-butyric acid has been studied.

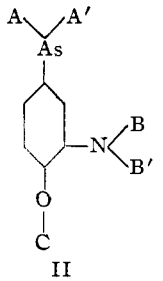
BERKELEY 4, CALIFORNIA RECEIVED DECEMBER 10, 1947

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS AND CO.]

3-Amino-4-hydroxybenzenearsonous Acid. II. Derivatives

BY C. K. BANKS, JOHN CONTROULIS, D. F. WALKER, E. W. TILLITSON,[1] L. A. SWEET AND O. M. GRUHZIT

The amine salts and arsenite hemiesters of 3-amino-4-hydroxybenzenearsonous acid (I, oxophenarsine) were described in the first paper of this series.[2] All of these compounds, as well as the dihalo derivatives, equilibrate readily in solution to a common ion, postulated to be 3-ammonium-4-hydroxybenzenearsonite. Since this nucleus has been unique in therapeutic agents for the treatment of spirochetal diseases, further variations of the general structure II have been made.

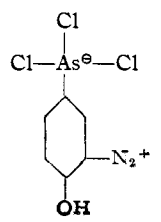


II

Previously reported compounds of this type include the aforementioned salts, hemiesters and acid adducts,[2] mercaptan derivatives in which A and A′ were mercaptoacetic acid, mercaptoacetamide and cysteine,[3] the acetyl derivative (B = $COCH_3$)[4] and compounds in which C was replaced by hydroxyalkyl[5–7] and alkyl groups.[8] Since variations in C appeared to be explored adequately, the principal variants studied were those of A and B. Since 3-amino-4-β-hydroxyethoxybenzenearsonous acid (III)[5] has been found to have practically the same *in vivo* spirochetal activity as I, the β-hydroxyethyl group was selected as the C variant for the study of multiple substitution.

The mercaptol derivatives of I were extended to include mercaptoacetone, octyl mercaptoacetate, thiomalic acid and unsubstituted sulfides. Hydrogen sulfide reacted with I to yield compounds having —As(SH)$_2$, —As(SH)(OH) and —AsS structures, depending on the conditions employed. The mercaptoacetic acid, octyl mercaptoacetate and mercaptoacetamide derivatives of III were also formed. The octyl mercaptoacetates were of interest in that they are soluble in oils.

The amine group was modified by substituting B and B′ with the acid succinamide, benzal, formaldehyde bisulfite and glucose bisulfite groups. Attempts to prepare the analogous formaldehyde sulfoxylate resulted in neoarsphenamine types. Similar amine derivatives were prepared in which A and A′ were replaced by thiols and where C was the hydroxyethyl group. The formaldehyde sul-



IV

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(2) Banks, *et al.*, THIS JOURNAL, **69**, 5 (1947).

(3) Barber, *J. Chem. Soc.*, 1020 (1929).

(4) Newbery and Phillips, *ibid.*, 2375 (1928).

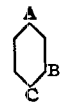
(5) Sweet and Hamilton, THIS JOURNAL, **56**, 2409 (1934).

(6) Bare and Hamilton, *ibid.*, **59**, 2444 (1937).

(7) Holcomb and Hamilton, *ibid.*, **61**, 1236 (1939).

(8) Doak, Steinman and Eagle, *ibid.*, **63**, 99 (1941).

TABLE I



	A	B	Yield, %	Empirical formula[a]	Analysis,[b] % Calcd.	Found	Toxicity[c] I. V.-white rats Compound LD50 mg./kg.	As, LD5 mg./kg.	As, LD50 mg./kg.	Trypanocidal activity[c] *T. equiperdum*-white rats M. Th. D. mg./kg.	M. C. D. mg./kg.	Th. I.	C. I.
1	As(OH)$_2$	NH_2	[d]	$C_6H_8AsNO_3$	34.51	34.47	19.5	17.5	6.73	0.6	2.0	32.5	9.7
2	As(OH)$_2$	NH_3Cl	[d]	$C_6H_9AsClNO_3$	29.54	29.62	21.0	16.0	6.21	.7	2.6	30.0	8.1
3	As(OH)(Cl)	NH_2	[d]	$C_6H_7AsClNO_2$	31.81	31.74	17.5	16.0	5.57	.6	2.2	29.1	8.0
4	AsO[e]	NH_3Cl	[d]	$C_6H_7AsClNO_2$	31.81	31.85	18.5	15.0	5.88	.6	2.5	30.8	7.4
5	[f]	NH_3Cl	[d]	$C_{14}H_{10}As_2Cl_2N_2O_5$	28.98	29.11	18.0	14.0	5.22	.4	2.0	45.0	9.0
6	AsO	$NH_3 \cdot 1/_2SO_4$	[d]	$C_{12}H_{14}As_2N_2O_8S$	30.20	30.08	17.5	14.0	5.29	.6	3.0	29.1	5.9
7	AsO	$NH_3 \cdot C_6H_9O_7$[g]	[d]	$C_{12}H_{16}AsNO_9$	19.05	18.98	33.0	20.0	6.29	2.0	4.0	17.5	8.3
8	AsO	$NH_3 \cdot C_6H_7O_7$[h]	[d]	$C_{12}H_{14}AsNO_9$	19.15	19.17	28.7	25.0	5.50	1.5	4.0	19.0	7.2
9	$AsCl_2$	NH_3Cl	[i]	$C_6H_7AsCl_2NO$	25.79	25.82	21.8	15.0	5.62	1.0	3.6	21.8	6.1
10	$AsBr_2$	NH_3Br	[j]	$C_6H_7AsBr_2NO$	17.68	17.71	32.5	20.0	4.78	1.5	5.0	21.6	6.5
11	As(SH)(OH)	NH_2	78	$C_6H_8AsNO_2S$	32.14	32.02[k]	...	..	...	..	..	..	...
12	As(SH)$_2$	NH_2	67	$C_6H_8AsNOS_2$	30.06	30.06	...	..	...	..	..	..	...
13	AsS	NH_2	55	C_6H_6AsNOS	34.83	34.58[l]	...	..	...	..	..	..	...
14	As(SH)(OH)	NH_3Cl	63	$C_6H_9AsClNO_2S$	27.79	27.74[m]	22.0	18.0	6.11	2.5	4.0	8.8	5.5
15	AsS	NH_3Cl	78	$C_6H_7AsClNOS$	29.77	29.98[n]	18.5	15.0	5.55	2.5	3.6	7.4	5.1
16	As(SCH_2CO_2H)$_2$	NH_2	92	$C_{10}H_{12}AsNO_5S_2$	20.51	20.48	...	..	...	..	..	..	...
17	As(SCH_2CO_2Na)$_2$	NH_2	87	$C_{10}H_{10}AsNNa_2O_5S_2$	18.31	18.46	18.0	16.0	3.30	0.8	3.0	22.5	6.0
18	As(SCH CONH$_2$)$_2$	NH_2	92	$C_{10}H_{14}AsN_3O_3S_2$	20.62	20.28[p]	...	..	...	..	..	..	...
19	As(SCH_2CONH_2)$_2$	NH_3Cl	90	$C_{10}H_{15}AsClN_3O_3S_2$	18.74	18.48[q]	32.5	20.0	3.75	3.0	4.0	10.8	8.2
20	As($SC_4H_6O_4$)$_2$[r]	NH_2	68	$C_{14}H_{16}AsNO_8S_2$	15.56	15.38	...	..	...	..	..	..	...
21	As($SC_{10}H_{19}O_2$)$_2$	NH_2	72	$C_{26}H_{44}AsNO_6S_2$	12.70	13.01	50.0	35.0	6.35	9.0	..	5.5	...
22	As(SCH_2COCH_3)$_2$	NH_3Cl	65	$C_{12}H_{17}AsClNO_3S_2$	18.83	19.01	...	..	...	..	..	..	...
23	AsO[t]	NO_2	78	$C_6H_4AsNO_4$	32.71	32.52	5.0	4.0	1.63	> 2.0	..	..	...
24	AsO	$NC_6H_5O_2$[u]	85	$C_{14}H_{11}AsNO_4$	22.55	22.40	25.0	20.0	5.64	2.0	8.0	12.5	3.1
25	AsO	$NHCOCH_3$	70	$C_8H_{10}AsNO_4$	28.91	28.75	5.0	4.0	1.45	3.0	> 3.0	1.7	...
26	AsO	$NHCH_2SO_3Na$[v]	90	$C_7H_9AsNNaO_6S$	22.49	22.25	20.0	12.5	4.50	10.0	15.0	2.0	1.3
27	As(OH)$_2$	$NHC_6H_{11}O_6$[w]	54	$C_{12}H_{16}AsNO_8$	19.76	19.52	...	..	...	..	..	..	...
28	As(OH)$_2$	$NHC_6H_{12}NaO_9S$[x]	55	$C_{12}H_{19}AsNNaO_{11}S$	15.50	15.21	70.0	60.0	10.85	>10.0	..	..	...
29	AsO	$NHC_4H_5O_3$[y]	82	$C_{10}H_{10}AsNO_6$	25.05	24.80	4.5	4.0	1.13	2.0	> 2.0	2.2	...
30	$AsCl_2$ -	N_2^+	92	$C_6H_4AsCl_2N_2O$	24.85	24.86	17.5	15.0	4.35	1.2	> 5.0	14.5	...
31	$AsCl_2(OH)$ -	N_2^+	54	$C_6H_6AsCl_2N_2O_2$	26.48	26.50[z]	...	..	...	..	..	..	...
32	As(SCH_2CO_2Na)$_2$	$NHCH_2SO_2Na$[aa]	70	$C_{11}H_{11}AsNNa_2O_7S_2$	14.71	14.78	30.0	25.0	4.41	5.0	10.0	6.0	3.0
33	As(SCH_2CO_2Na)$_2$	$NHCH_2SO_3Na$[v]	85	$C_{11}H_{11}AsNNa_2O_8S_2$	14.26	14.10	58.0	52.0	8.27	12.0	20.0	4.8	2.9
34	As(SCH_2CO_2Na)$_2$	$NHC_6H_{12}NaO_8S$[x]	68	$C_{16}H_{21}AsNNa_2O_{13}S_2$	11.09	10.84	101.0	93.0	11.20	12.0	15.0	8.4	6.7
35	As(SCH_2CONH_2)$_2$	$NHC_6H_{12}NaO_8S$[x]		[cc]			45.0	35.0	5.00	> 8.0	..	..	...
	C = OCH_2CH_2OH												
36	As(OH)$_2$	NH_2		[bb]			16.5	10.0	4.75	0.8	3.5	20.6	4.7
37	As(SCH_2CO_2H)$_2$	NH_2	82	$C_{12}H_{16}AsNO_6S_2$	18.30	18.25	...	..	...	..	..	..	...
38	As(SCH_2CO_2Na)$_2$	NH_2	90	$C_{12}H_{14}AsNNa_2O_6S_2$	16.53	16.48	27.5	25.0	4.55	2.5	3.5	11.0	7.9
39	As(SCH_2CONH_2)$_2$	NH_2	82	$C_{12}H_{18}AsN_3O_4S_2$	18.39	18.32	...	..	...	..	..	..	...
40	As(SCH_2CONH_2)$_2$	NH_3Cl	88	$C_{12}H_{19}AsClN_3O_4S_2$	16.88	16.70	60.0	40.0	10.13	3.0	8.0	20.0	7.5
41	As($SC_{10}H_{19}O_2$)$_2$[s]	NH_2	78	$C_{28}H_{48}AsNO_6S_2$	11.82	11.43	40.0	30.0	4.73	8.0	>20.0	5.0	...
42	As(OH)$_2$	$NHCH_2SO_3Na$[v]	85	$C_9H_{12}AsNNaO_7S$	19.86	19.72	50.0	30.0	9.93	15.0	20.0	3.3	2.5
43	As(SCH_2CO_2Na)$_2$	$NHCH_2SO_3Na$[v]	75	$C_{13}H_{18}AsNNa_2O_7S_2$	13.16	13.10	70.0	55.0	9.21	20.0	>30.0	3.5	...
44	As(SCH_2CONH_2)$_2$	$NHCH_2SO_3Na$[v]	67	$C_{13}H_{19}AsN_2NaO_7S_2$	14.31	14.20	27.5	25.0	3.84	3.0	7.0	9.2	4.0
45	As(SCH_2CO_2Na)$_2$	$NHC_6H_{12}NaO_8S$[x]		[cc]			75.0	60.0	7.50	8.0	30.0	9.4	2.5
	C = —$OCH_2CHOHCH_3$												
46	As(OH)$_2$	NH_2		[dd]			15.0	12.0	4.10	0.5	2.0	30.0	7.5
	C = —$OCH_2C(OH)(CH_3)_2$												
47	As(OH)$_2$	NH_2		[ee]			14.0	10.0	3.71	4.0	..	3.5	...

[a] No attempt has been made to indicate degree of polymerization but all —AsO and —AsS compounds are at least dimeric, see ref. 2. [b] By Banks and Sultzaberger, THIS JOURNAL, **69**, 1 (1947), when possible, otherwise by methods A or D, Banks, Sultzaberger, Maurina and Hamilton, *J. Am. Pharm. Assocn., Sci. Ed.*, **37**, 13 (1948). [c] See text for details. [d] Ref. 2. [e] Oxophenarsine hydrochloride, U. S. P. [f] Hydrochloride hemialcoholate of Ehrlich and Bertheim. *Ber.*, **45**, 756 (1912), in which the arsenic portion has the structure, HOAs(R)—O—As(R)OC_2H_5, see Ref. 2. [g] Ascorbate. [h] Citrate. [i] Dichlorophenarsine hydrochloride, U. S. P., Binz and Bauer, *Z. angew. Chem.*, **34**, 261 (1921). [j] U. S. Patent 2,222,384 (1940). [k] S, calcd.: 13.75%; found: 13.80%. [l] S, calcd.: 14.90%; found: 14.78%. [m] S, calcd.: 11.88%; found: 11.96%. [n] S, calcd.: 12.74%; found: 12.46%. [p] N, calcd.: 11.57%; found: 11.43%. [q] N, Calcd.: 10.51%; found: 10.30%. [r] From α-mercaptosuccinic (thiomalic) acid. [s] From octyl mercaptoacetate. [t] Christiansen, *et al.*, THIS JOURNAL, **47**, 2716 (1925). [u] Anil from vanillin. [v] Formaldehyde bisulfite. [w] Glucosyl. [x] Sodium glucosebisulfite. [y] Succinyl. [z] N, calcd.: 9.91%; found: 10.14%. [aa] Formaldehyde sulfoxylate. [bb] Sweet and Hamilton, *ibid.*, **56**, 2409 (1934). [cc] Used in solution without isolation. [dd] Stevenson and Hamilton, *ibid.*, **57**, 1600 (1935). [ee] Holcomb and Hamilton, *ibid.*, **61**, 1236 (1939).

foxylates of the thiol substituted arsenicals were prepared without difficulty.

I was also diazotized and 3-diazonium-4-hydroxybenzenetrichloroarsonite (IV) isolated on strong acidification. This proved to be identical with the reduction product of 3-diazonium-4-hydroxybenzenearsonate described by Schmidt and Hoffmann.[9] However, decomposition of this compound in methanol followed by precipitation

(9) Schmidt and Hoffmann, *Ber.*, **59**, 560 (1926).

with ether did not result in 3-diazido-3,4-quinone-1-dichloroarsine as they report but in 3-diazonium-4-hydroxybenzenedichloroarsonite. This was confirmed by elemental analysis for C, H, N, As and Cl, and by the reactions of the substance. The compounds prepared, their toxicity and trypanocidal activity are listed in Table I. The treponemacidal effect of selected compounds is given in Table II.

TABLE II

RELATIVE TREPONEMACIDAL ACTIVITY

No. in Table I	CD_{60}, mg./kg. × 3, I. V. rabbits	No. in Table I	CD_{60}, mg./kg. × 3, I. V. rabbits
1	1.5	17	1.5
2	1.0	33	4.0
3	1.0	35	5.25
5	1.5	36	1.5
9	1.5	46	1.75

Experimental

3-Amino-4-hydroxybenzenearsonous acid and 3-amino-4-β-hydroxybenzenearsonous acid were prepared according to previously published methods.[2,5]

Sulfides.—A solution of 3-amino-4-hydroxybenzenearsonous acid (0.1 mole), prepared by dissolving the acid in 100 ml. of water with an excess of hydrochloric acid and then neutralizing the solution to *p*H 6, was saturated with hydrogen sulfide. On standing in the refrigerator, the —As(SH)(OH) compound separated. When the addition of hydrogen sulfide was made in the presence of two equivalents of hydrochloric acid, the corresponding *hydrochloride* separated. On drying at 0.5 mm. and 37° over phosphorus pentoxide, the analogous —AsS compounds were formed. Addition of ammonium sulfide to the original solution precipitated the $As(SH)_2$ compound.

Mercapto Products.—Mercaptoacetic acid (0.2 mole) was added to a solution of the arsonous acid (0.1 mole) and the *p*H adjusted to 7 with sodium hydroxide. Seven volumes of alcohol were added to crystallize the *sodium salts*. The free *mercaptoacetic acid derivatives* were formed by acidification of aqueous solutions of their sodium salts with hydrochloric acid. Other mercapto derivatives were prepared by the reaction of an alcohol solution of the mercaptan with an alcohol solution of the arsonous acid. The *hydrochlorides* were crystallized by the addition of dry hydrogen chloride gas, with cooling, and the *free bases* liberated from aqueous solutions of the hydrochlorides by neutralization with sodium hydroxide.

Formaldehyde and Glucose Bisulfites.—Water (40 ml.) was heated to boiling for a few minutes, then allowed to cool. At 90° the sodium aldehyde bisulfite (0.1 mole) was added and when cooled to 70°, the arsonous acid, or other arylamino arsenical, was added (0.1 mole) and stirred until solution occurred. The solution was clarified with charcoal, filtered and cooled to 30° and 600 ml. of ethanol added. On standing in an ice-bath, the products crystallized.

Formaldehyde Sulfoxylates.—Water (40 ml.), previously boiled and cooled under nitrogen, was used to dissolve sodium formaldehyde-sulfoxylate (0.1 mole) and the arylaminoarsenical (0.1 mole). When the reaction was complete, the solution was clarified with charcoal and the product crystallized by the addition of six volumes of ethanol.

Acyl Derivatives.—The corresponding arsonic acids were reduced by previously described techniques.[2]

3-Diazonium-4-hydroxybenzenetrichloroarsonite.—3-Amino-4-hydroxybenzenearsonous acid (217 g.) was suspended in 60.0 ml. of 3 *N* hydrochloric acid. The solution was cooled externally and a mole of sodium nitrite, dissolved in water, added slowly. As soon as the diazotization was complete, the resulting solution was filtered and a liter of cold concentrated hydrochloric acid added slowly with cooling. The crystalline product was filtered off and washed with glacial acetic acid and ether.

The compound is a pale yellow powder, soluble in water, alcohols, acetone, and dioxane but insoluble in benzene, ether and petrolic ether. The compound exploded on heating. Solutions of the substance in water coupled with β-naphthol and α-dimethylaminonaphthalene to yield deeply colored dyes.

3-Diazonium-4-hydroxybenzenedichloroarsonite.—Ten grams of the trichloroarsonite was dissolved in a minimum of absolute methanol, allowed to stand a few minutes until gas evolution ceased and an excess of anhydrous ether added. A bright yellow product separated. It was filtered off, and dried in a vacuum desiccator. It has the same solubilities and reactions as the trichloro compounds.

Pharmacological Evaluations

Toxicity.—The intravenous toxicity of each compound was determined in albino male rats as previously described.[10] The dose which was lethal for 50% of the animals (LD_{50}) was calculated by the method of Dragstedt and Lang.[11] The LD_{50} and LD_5 are given in Table I in terms of mg. compound per kg. body weight of animal. The LD_{50} values in terms of compound administered also were converted to LD_{50} figures in terms of mg. As per kg. for purposes of comparison.

It can be seen that all of the simple salts and haloarsine derivatives of compound 1 have approximately the same toxicity in terms of arsenic administered (compound 10 may be considered an exception). This is compatible with the hypothesis that all derivatives of this type (1–10 incl.) are converted in aqueous solution to the same form.

The substitution of mercapto groups on arsenic (14, 15, 17, 19, 21, 32, 33, 34, 35) either did not affect or increased the relative toxicity. No general postulations can be made as to the result of substituting hydrogens of the amino group.

Trypanocidal Activity.—The compounds were administered to albino male rats previously infected with a standardized strain of *Trypanosoma equiperdum*. The techniques have been described previously.[10] The minimum therapeutic dose (M.Th.D.) is designated as the minimal dose of drug which entirely eliminated trypanosomes from the peripheral blood forty-eight hours after treatment and the minimum curative dose (M.C.D.) as that dose which prevented relapse of the infection for four weeks. The therapeutic index (Th.I.) is taken as the ratio LD_{50}/M.Th.D. and the curative index (C.I.) as the ratio LD_{50}/M.C.D. The results are given in Table I. Those compounds which can generate No. 1 on solution and neutralization all have comparable activities (2–10 incl.). Sulfhydryl derivatives were of the same or lower order of activity. In general, the trypanocidal effect was lessened when the amino group was substituted. While the substitution of the hydroxyl by a β-hydroxyalkoxyl group did not appreciably affect activity, further substitution on arsenic or nitrogen had approximately the same effect as was noted in the analogous hydroxyl compounds.

Treponemacidal Activity.[12]—Healthy normal rabbits of different breeds were infected with a virulent Nichols strain of *Treponema pallidum* and held until lesions became well developed. The rabbits were treated intravenously with selected compounds three times on alternate days. They were observed for regression of the lesions and by microscopic darkfield examination for the disappearance of spirochetes. The popliteal lymph glands of those rabbits showing no lesions three months after treatment were excised and implanted intratesticularly in normal

(10) Banks, Controulis, Tillitson and Gruhzit, THIS JOURNAL, **66**, 1771 (1944).

(11) Dragstedt and Lang, *J. Pharm. Exptl. Therap.*, **32**, 215 (1927–1928).

(12) A more complete discussion of procedures may be found in Gruhzit, O.M., *Arch. Derm. Syph.*, **32**, 848 (1935).

animals. Absence of syphilitic lesions in the transfer animals over a period of three months was taken as the criterion of cure. The results are given in Table II. In general, compounds which revert to 1 in solution had approximately equal activity, mercapto derivatives being about equally active. Substitution of hydroxyalkoxyl groups for hydroxyl may have decreased the activity slightly but substitution of amino hydrogen resulted in marked reduction of activity.

Summary

1. A number of new derivatives of 3-amino-4-hydroxybenzenearsonous acid have been prepared for trypanocidal and treponemacidal studies. These consisted of variations in which easily and difficultly hydrolyzable groups were attached to the arsenic, nitrogen and phenolic oxygen atoms.

2. Animal studies indicated that none of the compounds was more active than the parent compound and that only those compounds having readily hydrolyzed groups retained any appreciable activity.

DETROIT, MICHIGAN RECEIVED NOVEMBER 15, 1947

[CONTRIBUTION FROM THE INSTITUTE OF MATERIA MEDICA, NATIONAL ACADEMY OF PEIPING, SHANGHAI, AND THE PHARMACOLOGICAL LABORATORY, NATIONAL INSTITUTE OF HEALTH, NANKING]

Antimalarial Constituents of Chinese Drug, Ch'ang Shan, *Dichroa febrifuga* Lour

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A brief account on the isolation of an antimalarial alkaloid named dichroine from the Chinese drug, Ch'ang Shan, identified as *Dichroa febrifuga* Lour., has been reported.[1] Mention should be made that the name dichroine has been used previously by Hartwich[2] to indicate a carbohydrate of an indefinite nature isolated from the same plant. The alkaloid dichroine has the composition $C_{16}H_{21}O_3N_3$ and easily undergoes isomeric change under the action of heat, acids, and alkalies, and even with different solvents used. Three isomerides, which are provisionally named, α-, β- and γ-dichroines, have been obtained, melting, respectively, at 136, 145 and 160°, and being convertible into each other under suitable conditions. Oxidized with potassium permanganate, dichroine yields 4-quinazolone and some other products not yet identified. Hydrolysis with sodium hydroxide gives easily the decomposition products, anthranilic acid, formic acid, and ammonia, together with a compound which behaves like a pyrrole derivative. Benzoylation with benzoyl chloride furnishes most probably a tribenzoyl derivative of dichroine according to its nitrogen content. No presence of carboxyl-, methoxyl- and methylenedioxy- groups could be detected in the molecule of dichroine. Dichroine forms both normal and acid salts and a nitroso compound. Besides dichroine, 4-quinazolone, a base with the composition $C_{18}H_{23}N_3O_3$, and umbelliferon have also been isolated from the roots of Ch'ang Shan; the first one may be originally present in the plant or resulted during chemical manipulation. Synthetic quinazoline derivatives used as antimalarials have recently been investigated extensively by Magidson and Yolovchinskaya[3] and others. The isolation of 4-quinazolone from a natural plant affords a remarkable coincidence with the chemical research along this line, although the quinazolone nucleus has already been found in certain alkaloids.[4] Regarding the antimalarial activity of dichroines, the γ-isomeride shows the greatest, and α-isomeride the least; the curative dose for chicken malaria being found to be 4 mg. of γ-isomer per kg.[5]

(1) Chou, Jang, Fu, Kao and Huang, *Science (Chinese)*, **29**, No. 2, 49 (1947).

(2) Hartwich, *Neue Arzneidrog*, 127 (1897).

(3) Magidson and Yolovchinskaya, *J. Gen. Chem. (U. S. S. R.)*, **8**, 1797 (1938).

Experimental

The finely powdered root of Ch'ang Shan is percolated with 90% alcohol at room temperature for two days and the extract evaporated in a vacuum. The residue is taken up with dilute hydrochloric acid, filtered, and extracted repeatedly with ether, which constitutes fraction A. The acid solution is rendered slightly alkaline with sodium bicarbonate and shaken well with ether containing about 20% of chloroform (fraction B). The aqueous solution is then made strongly alkaline with potassium carbonate and extracted several times with chloroform (fraction C).

Umbelliferon, $C_9H_6O_3$.—The residue obtained from fraction A, by distilling off ether, crystallizes from alcohol in colorless needles, m. p. 224–227°, sparingly soluble in water, but easily soluble in chloroform, alcohol, and alkaline solutions, the last possessing an intense blue fluorescence. Its properties and analysis correspond well to umbelliferon (7-hydroxycoumarine). *Anal.* Calcd. for $C_9H_6O_3$: C, 66.6; H, 3.7. Found: C, 66.6; H, 3.9.

4-Quinazolone.—Fraction B, on evaporation of ether–chloroform mixture, gives a product which crystallizes from alcohol in silky long needles, m. p. 212–213°. It is identical in all respects with a sample of 4-quinazolone prepared by heating 2 g. of anthranilic acid and 1 g. of formamide for two hours at 120–130° and crystallizing the resulting products from alcohol. Its analysis as well as those of its hydrochloride and platinum salt confirms its composition $C_8H_6ON_2$. *Anal.* Calcd. for $C_8H_6ON_2$: C, 65.8; H, 4.1; N, 19.1. Found: C, 65.6; H, 4.4; N, 19.1.

Hydrochloride.—It is obtained by treating an alcoholic solution of 4-quinazolone with hydrochloric acid gas dissolved in alcohol and adding a sufficient quantity of ether; needles, m. p. 247°. Its aqueous solution is acid to litmus paper. *Anal.* Calcd. for $C_8H_6ON_2 \cdot HCl$: N, 15.3; Cl, 19.4. Found: N, 15.0; Cl, 19.2.

Platinum Salt.—It is obtained by treating an alcoholic solution of 4-quinazolone with an aqueous solution of platinum chloride in the presence of hydrochloric acid and

(4) Asahina, Manske and Robinson, *J. Chem. Soc.*, 1708 (1927).

(5) Jang and co-workers, private communication.