the α-ergostenol from yeast ergosterol as described by Reindel, Walter and Rauch.

2. The effect of a number of acid halides in causing isomerization of α-ergostenol to β-ergostenol has been investigated; no single factor could be made to account for isomerization in some cases and not in others.

3. A number of new esters of α- and of β-ergostenol are described.

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[CONTRIBUTION FROM THE DERMATOLOGICAL RESEARCH LABORATORIES]

AROMATIC AMIDES OF N-ARYLGLYCINE ARSONIC ACIDS

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In the search for therapeutic arsenical compounds, used in the treatment of syphilis, Jacobs and Heidelberger[1] deviated from the usual experimental lines, which involved changes in the aromatic nucleus containing arsenic. These older researches had involved changes in the structure of salvarsan base (diamino-di-hydroxy-arsenobenzene) and this field of study has been fairly well exhausted.

The above authors synthesized a new series of compounds utilizing the reaction between chloro-acetyl-alkyl or chloro-acetyl-aryl amines and arsanilic acid (*p*-aminophenylarsonic acid). The aromatic series furnished a new field for work inasmuch as the non-arsenical benzene nucleus could be still further substituted, giving rise to compounds of substituted chloro-acetyl-aryl amines and arsanilic acid. A large number of such compounds were prepared.

It is our purpose to add further to this series and in doing so we have used the chloro-acetyl derivatives of amines which in themselves are therapeutic to some degree. We have also obtained some compounds involving 3-methylarsanilic acid (*m*-methyl-*p*-amino-arsonic acid) which correspond to the known derivatives of arsanilic acid.

The intermediate compounds used by us in the preparation of the arsonic acids are chloro-acetyl derivatives of amines. In most of the experiments these were prepared by methods employed by Jacobs and Heidelberger;[2] the few exceptions will be described later.

Jacobs and Heidelberger in their experiments[3] employed arsanilic acid, and its corresponding derivatives, in the form of its sodium salt (*i. e.*, in alkaline solution). For several of our experiments this was not practicable inasmuch as the chloro-acetyl compounds were oxidizable in alka-

[1] Jacobs and Heidelberger, THIS JOURNAL, **41**, 1581 (1919).

[2] Jacobs and Heidelberger, *ibid.*, **39**, 1439 (1917); **41**, 458 (1919); *J. Biol. Chem.*, **20**, 686 (1915).

[3] Jacobs and Heidelberger, THIS JOURNAL, **41**, 1587 (1919).

line mediums and gave very dark colored products, this being especially the case with 5-chloro-acetylaminosalicylic acid.

In such cases we employed the free arsanilic acid (or derivative) in aqueous medium. This required more time for the reaction and in a few instances reduced the yield; nevertheless the product was purer in composition and much lighter in color. Then, again, according to Jacobs and Heidelberger,[4] the reaction between arsanilic acid and chloro-acetylanthranilic acid did not go in the desired sense entirely, due to the high reactivity of the chlorine atom in the chloro-acetyl compound, resulting in side reactions and an impure product. We considered that this was perhaps due to the presence of sodium hydroxide, so we employed only a water medium. The product we obtained showed by analysis that it was not exactly pure, but still it was practically so, and on further purification would finally have yielded an absolutely pure product.

All of the arsonic acids we prepared are crystalline products with the possible exception of the N-(phenyl-4-arsonic acid)-glycyl-acriflavine, which is a very fine amorphous powder.

The subsequently mentioned compounds were elaborated with a view to possible application in the treatment of syphilis. The major requirements for qualification of a chemical substance as a therapeutic agent are therapeutic efficiency and relatively low toxicity, allowing a large margin of safety between the therapeutic and the toxic doses. From the point of view of toxicity some of our preparations showed remarkably high qualities. When injected into rabbits they proved to be of much lower toxicity than other pentavalent arsenicals, including tryparsamide and stovarsol (acetarsone), both accepted antisyphilitic drugs and generally considered of low toxicity.

TOXICITY OF VARIOUS ORGANIC COMPOUNDS OF ARSENIC WHEN ADMINISTERED INTRAVENOUSLY TO RABBITS

Compound	Max. tolerated dose calcd. per kg. of body weight, g.	Lethal dose, g.
Arsanilic acid	0.08	0.1
Tryparsamide (sodium N-phenylglycine-amide-*p*-arsonic acid)	1	1.25
Stovarsol (3-acetyl-amino-4-hydroxyphenylarsonic acid)	0.1	0.12
Sodium salt of stovarsol	0.5	0.6
Arsonodiacetyl-(diacetyl-3,5-diamino-4-hydroxy-phenyl-arsonic acid)	0.8	1
N-(phenyl-4-arsonic acid)-glycyl-5′-amino-salicylic acid	0.9	1
N-(phenyl-4-arsonic acid)-glycyl-5′-amino-salicylic acid (sodium salt)	1.75	2
N-(phenyl-2-methyl-4-arsonic acid)-glycyl-5′-amino-salicylic acid	0.9	1

[4] Jacobs and Heidelberger, THIS JOURNAL, 41, 1627 (1919).

Thus, N-(phenyl-4-arsonic acid)-glycyl-5′-aminosalicylic acid (sodium salt) is considerably less toxic for rabbits than tryparsamide and the sodium salt of stovarsol. The results of the spirocheticidal tests of this compound, however, were not very encouraging.

RESULTS OF INTRAVENOUS INJECTIONS OF N-(PHENYL-4-ARSONIC ACID)-GLYCYL-5′-AMINO-SALICYLIC ACID INTO RABBITS INOCULATED WITH TREPONEMA PALLIDUM

Dose per kilo of body weight, g.	Dark field negative for spirochetes after	Testicles normal after	Testicles remained normal (last examination)	Results of lymph node transplantation into control animals
0.15	14 days	Animal died after 3 days		
0.15	26 days	61 days	116 days	Negative
0.3	9 days	48 days	70 days	Negative

While this compound, as seen from the above table, is capable of destroying spirochetes in the animal body, it is evident that a dose had to be employed which was quite near to the maximum tolerated, in order to obtain early negative results in the dark field examination. It took nine days to make spirochetes disappear from lesions when the dose of 0.3 g. was used, *i. e.*, one-third of the maximum tolerated dose. For comparison, it was found by us that only 0.015 g. (about one-eighth of the maximum tolerated dose) of arsphenamine was required to obtain the same result in one day.

The same compound did not prove to be of therapeutic value in experimental trypanosomiasis in albino rats when injected intravenously in the dose of 1.1 g. per kilo (rats tolerated it in as high a dose as 4.5 g.).

Experimental Part

Preparation of Chloro-acetyl Amines.—The general method for chloro-acetylation is as follows: 1 mole of the amino compound or hydrochloride is dissolved or suspended in 1 liter of ice and water and cooled in a bath of ice water. Then with good stirring 1.5–2 moles of chloro-acetyl chloride is slowly added. The chloro-acetyl compound separates and is filtered. It is then washed with water or 2% hydrochloric acid, except in the cases of chloro-acetylamino-antipyrine and chloro-acetylacriflavine, which are both somewhat soluble in water. With the exception of these two compounds, all can be recrystallized from water.

TABLE I

CHLORO-ACETYL COMPOUNDS

( )-Chloro-acetyl	Formula	M. p., °C.	Nitrogen, % Calcd.	Found
4-( )-aminobenzoic acid	*p*-$(CH_2ClCO)NHC_6H_4COOH$	248	6.56	6.55
2-( )-(4-nitro)-toluidine	$ClCH_2CONHC_6H_3(NO_2)CH_3$-(*p,o*)	151	12.25	12.08
5-( )-aminosalicylic acid	$ClCH_2CONHC_6H_3(OH)COOH$-(*p,o*)	242–244	6.10	6.19
( )-amino-antipyrine	$ClCH_2CONHC_{11}H_{11}N_2O$	187	Cl, 12.66	12.87
( )-acriflavine	$ClCH_2CONHC_{14}H_{12}N_2Cl$	215–220, d.	.....	...
4-( )-aminoguaiacol	$ClCH_2CONHC_6H_3(OCH_3)OH$-(*m,p*)	117	6.48	6.29

Preparation of Arsonic Acid Amides in Alkaline Medium.—The method used was that of Jacobs and Heidelberger in which the arsonic acid is dissolved in alkali to give the sodium salt in solution, except that we used 2 moles of the arsonic acid instead of 1 mole.

Two moles of arsanilic acid (or 3-methylarsanilic acid) was dissolved in a volume of normal sodium hydroxide solution (2 moles) and to this was added 1 mole of the chloro-acetyl amine. The mixture was then heated under a reflux condenser for a time, varying with each reaction. Usually after refluxing for one to two hours the solid matter goes into solution, and on further heating precipitates again. In some cases the solid matter never goes into solution. The reaction is usually finished when a test portion of the mixture is completely soluble in alkali. The mixture is then made acid to congo red with hydrochloric acid and filtered off. The crude arsonic acid amide is insoluble. This can be purified by washing well with dilute hydrochloric acid and water, then dissolving in dilute sodium hydroxide, treating with "nuchar" and reprecipitating with hydrochloric acid while hot. The purified amides are insoluble in hot or cold water, insoluble in organic solvents, insoluble in dilute acids but soluble in dilute alkali. The following amides were prepared in this manner.

TABLE II

ARSONIC ACID AMIDES FROM ALKALINE MEDIUM

Compound	M. p., °C.	Time of reaction, hours
N-(Phenyl-4-arsonic acid)-glycyl-4′-aminobenzoic acid	Darkens at 230, melts at 260–265 (dec.)	3
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-2′-toluidide	246 (dec.)	5
N-(Phenyl-4-arsonic acid)-glycine-(4′-nitro)-*o*′-toluidide	115–120 (dec.)	6
N-(Phenyl-4-arsonic acid)-glycyl-amino-antipyrine	270 (dec.)	6
N-(Phenyl-4-arsonic acid)-glycyl-4′-aminoguaiacol	215–217	5

Formula	Nitrogen, % Calcd.	Found	Arsenic, % Calcd.	Found
p-$H_2O_3AsC_6H_4NHCH_2CONHC_6H_4COOH$-*p*	7.08	6.90	19.00	18.55
o,*p*-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_4CH_3$-*o*	7.36	6.93	19.80	20.01
p-$H_2O_3AsC_6H_4NHCH_2CONHC_6H_3(NO_2)CH_3$-*p*,*o*	10.24	10.04	18.35	17.86
p-$H_2O_3AsC_6H_4NHCH_2CONHC_{11}H_{11}N_2O$	..	..	16.24	15.90
p-$H_2O_3AsC_6H_4NHCH_2CONHC_6H_3(OCH_3)OH$-*m*,*p*	7.07	6.82	18.93	19.19

Preparation of Arsonic Acid Amides in Aqueous Medium.—This method is practically the same as the previous one with the omission of the alkali. The reactions require longer heating but the yields are approximately equal to those in reactions employing sodium hydroxide. The yields in both cases range from 25–40% based on the chloro-acetyl compound.

TABLE III

ARSONIC ACID AMIDES FROM AQUEOUS MEDIUM

Compound	M. p., °C.	Time of reaction, hours
N-(Phenyl-2-methyl-4-arsonic acid)-glycyl-4′-aminobenzoic acid	Dec.	3
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-anilide	>275	6
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-(4′-nitro)-*o*′-toluidide	285–286 (dec.)	10
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-*p*′-amino-acetanilide	.....	40

TABLE III (*Concluded*)

Compound	M. p., °C.	Time of reaction, hours
N-(Phenyl-4-arsonic acid)-glycyl-5'-aminosalicylic acid	Darkens at 190, melts at 230–235 (dec.)	8
N-(Phenyl-2-methyl-4-arsonic acid)-glycyl-5'-aminosalicylic acid	240–245 (dec.)	10
N-(Phenyl-4-arsonic acid)-glycyl-acriflavine	Darkens at 240, does not melt below 300	6
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-β'-naphthylamide	260–262 (dec.)	8
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-α'-naphthylamide	254–255 (dec.)	8
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-piperidide	.....	12
N-(Phenyl-2-methyl-4-arsonic acid)-glycine-benzyl amide	>275	15
N-(Phenyl-2-methyl-4-arsonic acid)-glycyl-anthranilic acid	.....	6

Formula	Nitrogen, % Calcd.	Found	Arsenic, % Calcd.	Found
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_4COOH$-*p*	6.84	6.34	18.33	18.10
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_5$	7.67	7.64	20.54	20.87
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_3(NO_2)CH_3$-*p,o*	9.90	9.52	17.68	17.20
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_4NHCOCH_3$-*p*	9.95	9.52	17.77	18.04
p-$H_2O_3AsC_6H_4NHCH_2CONHC_6H_3(OH)COOH$-*p,m*	6.83	6.55	18.29	18.05
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_3(OH)COOH$-*p,m*	6.09	6.04	16.54	16.14
p-$H_2O_3AsC_6H_4NHCH_2CONHC_{14}H_{12}N_2Cl$	..	..	10.83	10.72
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_{10}H_7$-$\beta$-	6.74	6.43	18.07	18.48
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_{10}H_7$-$\alpha$-	6.74	6.37	18.07	18.45
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONC_5H_{10}$	..	..	21.00	21.33
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHCH_2C_6H_5$	7.38	7.13	19.78	20.10
o,p-$CH_3(H_2O_3As)C_6H_3NHCH_2CONHC_6H_4COOH$-*o*	6.84	6.37	18.33	17.82

Two moles of arsanilic acid (or 3-methylarsanilic acid) is suspended in one liter of water and 1 mole of chloro-acetylamino compound added. The mixture is then heated until a test portion shows complete solubility in alkali. In some of the reactions the insoluble matter does not go completely into solution and the reaction is very slow, requiring long heating, as is particularly the case with *p*-chloro-acetylamino-acetanilide, which is heated for forty hours. After acidification with hydrochloric acid, the product is filtered and washed. It is purified by using "nuchar" and reprecipitating from alkaline solution. The amides obtained by this method are also insoluble in all solvents except dilute alkali.

Summary

1. Some new arsonic acids have been prepared following the work of Jacobs and Heidelberger.

2. The preparation of intermediate chloro-acetyl compounds has been described.

3. Some of the preparations described proved to be valuable, being of low toxicity. Their therapeutic effect, however, was found to be low.

PHILADELPHIA, PENNSYLVANIA