and nitrogen but not for carbon. Hydrogen sulfide was liberated in some cases but no free sulfur was produced.

Ten of the pure strains of yeasts attacked potassium thiocyanate with the liberation of hydrogen sulfide. *Mycoderma vini, Mycoderma monosa, Mycoderma lactis* and Brewers' yeast formed especially large amounts of hydrogen sulfide from this compound. Nagaeli has reported that ammonium thiocyanate was unsuitable as a source of sulfur for yeasts.

Thiourea.—A 3% solution was used. Fourteen of the yeasts formed hydrogen sulfide from it. Nagaeli has reported that this urea was unsuitable as a source of sulfur for yeasts.

Free Sulfur.—About 0.2 g. of Merck's resublimed sulfur was placed in the bottom of each culture tube, to which were added about 10 cc. of the special culture medium. After sterilization, this sulfur collected in the bottom of the culture tubes in the shape of a button but available for the yeasts. Eight of the strains formed no hydrogen sulfide from free sulfur. In each of these 8 tubes there was good evidence of vigorous growth.

Summary.

The budding fungi used in this investigation are able to reduce the sulfur in cystine to hydrogen sulfide. With regard to peptone this characteristic is less extensive. Most of the strains were able to attack the sulfur linkage in thiosulfate to produce hydrogen sulfide. Thiosulfates are probably reduced to sulfite and hydrogen sulfide. Contrary to statements in the literature that yeasts are unable to reduce sulfates, 10 of the strains used in this investigation reduced sodium sulfate to hydrogen sulfide. A few of the yeasts reduced sodium sulfite. Sodium taurocholate was reduced to hydrogen sulfide by 2 strains, while sodium phenolsulfonate was not, although there was good growth in this latter substrate. Potassium thiocyanate and thiourea were also reduced. Hydrogen sulfide was also formed by many of the yeasts from free sulfur. Yeasts seem to be able to split some of the more stable linkages of sulfur, a characteristic which is probably not so wide spread among the bacteria.

ANALOGUES OF ATROPINE AND HOMATROPINE.

By Louis F. Werner.

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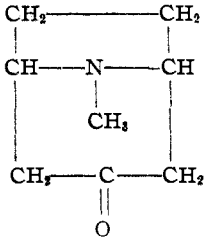
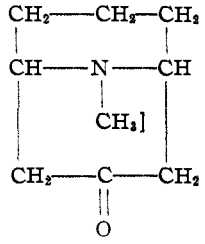
The alkamine tropine, one of the hydrolysis products of atropine, is changed upon oxidation to the ketone tropinone. This ketone is closely related to another ketone known as pseudopelletierine, which occurs naturally in the bark of the pomegranate tree. The relationship between the two is shown by the following structural formulas:



Tropinone.



Pseudopelletierine.

As can be readily seen, pseudopelletierine differs from tropinone in that it has one carbon atom and two hydrogen atoms more than tropinone.

Tropinone upon reduction is converted into two stereoisomeric alcohols. Upon being reduced electrolytically, or with concentrated hydriodic acid and zinc dust, it is reduced to tropine, but if reduced with sodium and alcohol it yields pseudotropine, a stereoisomer of tropine. These two stereoisomers have different physical properties, and the esters of these alcohols with the hydroxy acids have different physiological properties.

Pseudopelletierine upon reduction also gives two stereoisomeric compounds. Ciamician and Silber[1] found that an alkamine was formed upon reducing pseudopelletierine with alcohol and sodium, which they named methylgranatoline. The author found that a stereoisomer is formed by reducing pseudopelletierine with concentrated hydriodic acid and zinc dust, which may therefore be called iso-methylgranatoline. These two isomers have different physical properties, as is the case of the two reduction products of tropinone.

As was stated, an important difference in the properties of the reduction products of tropinone lies in the different physiological activities of the esters of the hydroxy acids with these alcohols. Thus the tropic acid ester (atropine), and the mandelic acid ester (homatropine) of tropine have strong mydriatic power, whereas the tropic acid esters and the mandelic acid ester of pseudotropine are devoid of such properties.

The object of the work undertaken was: To prepare the second reduction product of pseudopelletierine which was theoretically possible, and then to determine any difference in the physiological activities of the tropic acid and mandelic acid esters of these two stereoisomers.

The hydrobromides of the mandelic acid and tropic acid esters of isomethylgranotoline were prepared, and were found to have no mydriatic powers. These two artificial alkaloids were not obtainable in a crystalline condition, it being necessary to isolate them, and purify them in the form of their hydrobromides.

The hydrobromides of the mandelic acid and tropic acid esters of methylgranatoline were also prepared, and were found to have strong mydriatic

[1] *Ber.*, **26**, 2740 (1893).

properties. The hydrobromide of the tropic acid ester was obtained as a crystalline powder, but the hydrobromide of the mandelic acid ester was obtained as a syrup only.

From the viewpoint of physiological activity alone, tropine and methylgranatoline should constitute one type of stereoisomer, that is the type whose hydroxy acid esters have mydriatic power, whereas pseudotropine and iso-methylgranatoline are another type of isomer whose hydroxy acid esters are lacking in mydriatic powers.

From the standpoint of physical properties we would predict different results from those found above, as we would naturally group tropine (m. p. 63°) and iso-methylgranatoline (m. p. 65°) together and pseudotropine (m. p. 108°) and methylgranatoline (m. p. 100°) together. From the analogy in the methods of preparation of tropine and iso-methylgranatoline, and of pseudotropine and methylgranatoline we would classify these compounds into these two groups also. Thus methylgranatoline is prepared by reducing pseudopelletierine with alcohol and sodium, and pseudotropine is prepared from the corresponding ketone tropinone in analogous manner: iso-methylgranatoline and tropine are both prepared from the corresponding ketones by reduction with zinc dust and hydriodic acid. These results show the uncertainty of associating similarity of chemical and physical properties of compounds with similarity in physiological behavior.

Experimental.

Preparation of Methylgranatoline.—The method used by Ciamician and Silber,[1] slightly modified, was followed. Ten g. of pseudopelletierine were dissolved in 200 cc. of ethyl alcohol, and metallic sodium added until no more would dissolve, the reaction being carried out in a flask heated by a metal bath. Water was then added, and the alcohol distilled off, and the aqueous alkaline residue made acid with hydrochloric acid. This acid solution was then saturated with sodium bicarbonate, and the unchanged pseudopelletierine removed by shaking out with chloroform. The solution was then made strongly alkaline with sodium hydroxide solution, and the methylgranatoline shaken out with chloroform. Upon drying the chloroform solution over anhydrous sodium sulfate, the methylgranatoline was obtained as a crystalline solid upon distilling off the chloroform. Upon recrystallizing from petroleum ether, the pure compound is secured, melting at 100° (uncorr.).

Preparation of Iso-Methylgranatoline.—The method used was analogous to that used by Willstaetter[2] in reducing topinone to tropine. Ten g. of pseudopelletierine were dissolved in 120 g. of conc. hydriodic acid (sp. gr., 1.96), and was then cooled to 0°. Twenty g. of zinc dust were then added,

[1] *Ber.*, **26**, 2740 (1893).

[2] *Ibid.*, **33**, 1174 (1900).

stirring constantly, and the temperature was not allowed to rise above 0°. The reaction required about 2 hours. The mixture was allowed to stand for 24 hours, and then an excess of sodium hydroxide solution added, and the mixture shaken out with chloroform. This chloroform solution contained both pseudopelletierine and the new alcohol. In order to separate the two, the chloroform solution was shaken out with an excess of dil. hydrochloric acid, and after separating from the chloroform, the acid solution was saturated with sodium bicarbonate. This solution was then shaken out with chloroform to remove the pseudopelletierine, and then made strongly alkaline with sodium hydroxide solution, and the iso-methylgranatoline shaken out with chloroform. After being dried over anhydrous sodium sulfate, the alkamine is obtained as a crystalline residue upon removing the chloroform. It was then recrystallized from petroleum ether. The base is readily soluble in water, alcohol, and chloroform, but less soluble in petroleum ether. M. p. 65° (uncorr.).

Subst., 0.2515; N, 20.45 cc. at 761 mm. and 21°; collected over 30% KOH solution.

Calc. for $C_9H_{17}NO$: N, 9.032%. Found: 9.310%.

Preparation of the Hydrobromide of Tropic Acid Ester of Iso-Methylgranatoline.—Three g. of iso-methylgranatoline and 3 g. of tropic acid were dissolved in 10% hydrochloric acid, and the mixture heated for 70 hours on a water bath, the acid being replaced from time to time as it evaporated. The mixture was then diluted with water, and the excess tropic acid removed by shaking out with ether. Ammonia in excess was then added to the aqueous solution, and the ester shaken out with a little chloroform. The chloroform solution was extracted then with dilute hydrobromic acid, avoiding an excess. This aqueous solution of the hydrobromide was evaporated to dryness in a vacuum desiccator over conc. sulfuric acid. The dry residue was then recrystallized from absolute ethyl alcohol. It was found necessary to repeat the recrystallization several times to completely remove all ammonium bromide. The product so obtained was a white crystalline powder, turning yellow upon prolonged exposure to the light. It is soluble in water or hot alcohol, less so in cold alcohol. M. p. 233° (uncorr.).

Subst., 0.3909; N, 13.20 cc. at 757 mm. and 26°; collected over 30% KOH solution.
Calc. for $C_{18}H_{26}NO_3Br$: N, 3.64%. Found: 3.76%.

Preparation of the Hydrobromide of the Mandelic Acid Ester of Iso-methylgranatoline.—Three g. of iso-methylgranatoline and 3 g. of mandelic acid were dissolved in 10% hydrochloric acid, and were heated for 70 hours in an evaporating dish upon a water bath. The hydrochloric acid which evaporated was replaced, from time to time, by the addition of new acid. Only sufficient acid was added to keep all the reacting products in solution. At the end of the time stated, the reaction mass was diluted with a little

water, and the excess mandelic acid removed by shaking out with ether. The alkaloid was liberated by adding ammonia water in excess and was then taken up with a little chloroform. The hydrobromide was secured from this chloroform solution by shaking out with dil. hydrobromic acid, and evaporating the aqueous solution in a vacuum desiccator over sulfuric acid. The pure hydrobromide is secured by recrystallizing the crude salt from absolute alcohol. M. p. 229° (uncorr.). The salt is readily soluble in water or hot alcohol, slightly soluble in cold alcohol.

Subst., 0.4155; N, 14.50 cc. at 763 mm. and 25°; collected over 30% KOH solution.

Calc. for $C_{17}H_{24}NO_3Br$: N, 3.78%. Found: 3.93%.

Preparation of the Hydrobromide of the Tropic Acid Ester of Methylgranatoline.—Four g. of methylgranotoline and 4 g. of tropic acid were heated for 70 hours in an evaporating dish on a water bath with 0.25% hydrochloric acid. Sufficient acid was added from time to time, to keep all the reacting substances in solution. At the end of this time the mixture was acidified with conc. hydrochloric acid, and the unchanged tropic acid removed with ether. The aqueous solution was then made alkaline with ammonia water, and the base taken up with chloroform. The hydrobromide was formed by extracting the alkaloid from the chloroform by shaking out with dilute hydrobromic acid. Upon evaporating this aqueous solution in a vacuum desiccator over sulfuric acid, the crude hydrobromide was secured, which was purified by recrystallization from absolute alcohol. M. p. 220° (uncorr.). The salt is readily soluble in water and hot alcohol, slightly soluble in cold alcohol.

Subst., 0.4209; N, 14.10 cc. at 759 mm. and 20°; collected over 30% KOH solution.

Calc. for $C_{18}H_{26}NO_3Br$: N, 3.64%. Found: 3.84%.

Preparation of the Hydrobromide of the Mandelic Acid Ester of Methylgranatoline.—This compound was made by heating 4 g. of mandelic acid and 4 g. of methylgranatoline with 0.25% hydrochloric acid in sufficient quantity to keep all the reacting substances in solution. After heating for 70 hours, the mixture was acidified with hydrochloric acid, and excess mandelic acid removed by shaking out with ether. The aqueous solution was then made alkaline with ammonia water, and the ester shaken out with chloroform. The hydrobromide was secured as a viscous syrup upon extracting this chloroform solution with dil. hydrobromic acid, and evaporating *in vacuo* over sulfuric acid. The sulfate and the hydrochloride were also made, but these also were obtained as syrups only. No analysis was attempted of these viscous products.

Conclusion.

The results of the work show that, analogous to tropinone, pseudopelletierine gave two stereoisomers upon reduction. Similar to the two isomers

secured by the reduction of tropinone, one of these isomers when converted to the tropic or mandelic acid ester had no mydriatic properties, whereas the tropic and mandelic acid esters of the other had strong mydriatic effects. Use of the physical properties and methods of formation of these isomers was shown to be unreliable as a method of predicting their probable physiological effects.

The author wishes to express his gratitude to the Cincinnati Board of Health, in whose laboratory he was given the opportunity to determine the physiological effects of these alkaloids.

CINCINNATI, OHIO.

[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF YALE UNIVERSITY.]

THE RELATIVE STABILITY OF HALOGEN SUBSTITUTED ALIPHATIC ACIDS IN WATER SOLUTION.

II. THE PROPIONIC ACID AND BUTYRIC ACID SERIES.

BY G. S. SIMPSON.

Received January 21, 1918.

In a recent paper from this laboratory[1] the relative stability of halogen substituted acetic acids as determined by the hydrolysis of their sodium salts was discussed. This article describes the result of a similar investigation of the stability of several halogen substituted acids in the propionic and butyric acid series.

Preparation of Materials.

The ethyl esters of α-bromobutyric acid and α-bromoisobutyric acid, which were in stock, were readily purified by distillation. All other acids or esters were prepared as described below.

α-Chloropropionic ester was made from lactic acid by the method of Loven.[2] Dried calcium lactate was allowed to react with phosphorous pentachloride and the α-chloropropionyl chloride obtained was added to absolute alcohol to form the ester. After purification the product boiled at 145–147°.

α-Bromopropionyl bromide was made by adding bromine to dry propionic acid in the presence of red phosphorus according to Zelinsky's[3] method. This was added to absolute alcohol to form α-bromopropionic ester which, after purification, boiled at 157–159°.

β-Iodopropionic acid was prepared by heating iodine and yellow phosphorus with an aqueous solution of glyceric acid, obtained by the oxidation of glycerine with fuming nitric acid and purification by means of its calcium salt. This method of purification of glyceric acid, suggested by

[1] Drushel and Simpson, THIS JOURNAL, **39**, 2453 (1917).

[2] *J. prakt. Chem.*, [2] **29**, 367 (1884).

[3] *Ber.*, **20**, 2026 (1887).