to the precipitate, certain minor adaptations were necessary, the details of which will not be discussed here. The presence of rhein, kaempferol, aloe-emodin and organic acids in the sediment of fluidextract of senna was indicated. These are also to be found in the crude drug.

A water-insoluble resinous substance was obtained from an alcoholic extract of the precipitate by subjecting the latter to steam distillation. In quantity it represented about one-sixth of the total solids extracted with alcohol.

About fifty per cent of this resinous substance was soluble in potassium hydroxide solution, the solution yielding a red color, characteristic of anthraquinone derivatives. According to its solubility in potassium hydroxide, this substance should consist of about one-half "acid resins," the remainder being "indifferent resin" or "resin anhydride." The alkali insoluble portion of this resinous mass was quite readily soluble in nitric acid.

THE PRECIPITATE FROM THE BORAX-TREATED SAMPLES OF THE FLUIDEXTRACT OF SENNA.

The precipitates from the borax-treated samples were subjected to solvent, and other tests. The results are summarized as follows:

1. The "borated" precipitates yielded about three per cent more alcohol-soluble extractive than the ordinary precipitates.
2. The "borated" precipitates yielded much more extraneous amorphous material than the regular precipitates. This made it hard to test for the constituents, but anthraquinone substances were in evidence.
3. Upon being subjected to steam distillation the "borated" precipitates were found to yield much less insoluble residue than the usual precipitates. The residue was not soluble in nitric acid and gave the hydroxyanthraquinone reaction.

REFERENCES.

(1) Tutin, F., *Trans. Chem. Soc.*, 103, 2006 (1913).
(2) Dragendorff, G., "Plant analysis," page 8, translated by H. G. Greenish.

NEW IODINE—BILE COMPOUND (IODOCHOLEATE).*

BY PAUL GOEDRICH.[1]

THE PHYSICAL AND CHEMICAL PROPERTIES, GERMICIDAL AND PHARMACOLOGIC ACTION.

At the time that iodine was introduced to medicine as a germ-killing agent its toxic properties were known, and they caused very much concern. This situation has not changed very much.

The destructive action of iodine to the broken and unbroken skin, especially when covered by cloths or bandages, limits its field largely to First Aid; also in preparing the operative field, iodine must be carefully removed by alcohol to avoid tissue necrosis.

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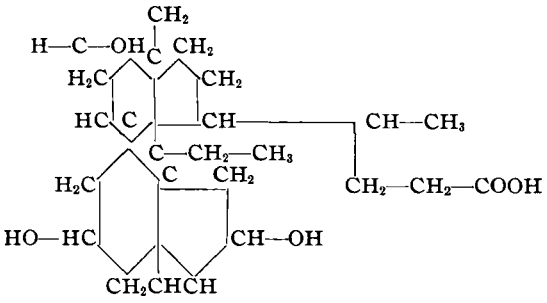
Iodine in the form of tincture and Lugol Solution is more than 100 years old, and all efforts to improve it have been limited.

In the course of experimentation on this subject, the author found a very surprising affinity of bile acids for iodine. In the natural bile these acids are present in the form of the sodium salt of the glycocholic and taurocholic acid, and so-called paired cholic acids.

CHART A.



Taurocholic Acid

$C_{20}C_{33}(CH—OH)_3—COOH—CH_2—SO_3H$

Cholic Acid — $CH—NH_2$

Amino-ethyl-sulfonic acid (Taurin)

Glycocholic Acid

$C_{20}H_{33}(CH—OH)_3—COOH—CH_2—COOH$

NH_2

Amino-acetic acid (Glycocol)

$(C_{26}H_{45}NSO_7)I_3$—Iodotaurocholic Acid

$(C_{26}H_{43}O_6N)I_3$—Iodoglycocholic Acid

It appears that these bile acids are compounds of the cholic acid with two different amino acids—the Glycocoll (Aminoacetic Acid) in case of the glycocholic acid, and the Taurin (Aminoethylsulphonic Acid) in the case of the taurocholic acid.

Until now it is believed that strong water solutions of iodine can only be made by addition of iodides. The choleates offer a new vehicle to obtain highly concentrated water solutions of iodine.

The bile salt, even in dry state, immediately forms a black iodine-like adsorption compound with the added iodine, which is soluble in water without any addition of iodides.

The question now arises whether the cholic acid alone would not also form a similar compound with iodine. This question is to be answered with yes and no. Cholic acid will form an adsorption compound with iodine, as was found a long time ago by Mylius and Kuester (1), but this compound is blue, unstable and not soluble in water, and it did not find any practical use. This indicates that the amino acid content of the natural bile acid has an important rôle in the formation of these compounds.

The complete adsorption takes place in proportion of one molecule of glycocholic acid to three atoms of iodine; if less glycocholic acid is used, free iodine appears in an ether extraction. The taurocholic acid forms the iodine compound in the same proportion. This, as far as our present knowledge goes, gives the iodoglycocholic acid the formula ($C_{26}H_{43}O_6N$) I_3 and the iodotaurocholic acid the formula ($C_{26}H_{45}NSO_7$) I_3. These compounds are soluble in water and also in alcohol, but not in ether, chloroform, carbon disulfide, etc. They give the blue starch reaction and are titrable with sodium thiosulphate.

CHART B.—IODINE ADSORPTION BY 5 CC. NORMAL HORSE SERUM.

Quantity of Used Iodine Solution.	Titrable Iodine Content.	Time of Exposure.	Starch-Iodine Reaction after Experiment.	Quantity of Iodine Recovered.
0.2 cc. U. S. P. Tincture	2.1%	5 min.	−	−
0.2 cc. Iodocholeate Solution	2.1%	5 min.	+	50%
0.5 cc. U. S. P. Tincture	2.1%	55 min.	−	−
0.5 cc. Iodocholeate Solution	2.1%	55 min.	+	50%
0.3 cc. U. S. P. Tincture	3.26%	10 min.	−	−
0.3 cc. Iodocholeate Solution	3.26%	10 min.	+	60%

An interesting feature of these compounds is that they combine much less with organic matter than tincture or Lugol's Solution, as Chart B indicates. The significance of this property will be seen in the bacteriologic and pharmacologic action. It might also be stated that steel and rubber are also very much less affected by the iodocholeates than by the tincture of Lugol's Solution. Among the physical properties of this product the most interesting one is the low volatility. It is well known that the tincture of iodine loses its iodine content rapidly after exposure, especially at body temperature and upon heating.

Iodocholeate solutions in water can be concentrated in open containers on the steaming water-bath, practically without any loss of Iodine.

CHART C.—CONCENTRATION TEST ON IODOCHOLEATE, TEMPERATURE 80° C.

Titrable Iodine Content before Concentration.	Original Quantity.	Concentrated to.	Iodine Content after Concentration.
1.48%	100 cc.	50 cc.	2.96%
2.1%	100 cc.	50 cc.	4.01%
3.02%	100 cc.	50 cc.	5.87%
4.1%	100 cc.	50 cc.	8.05%

To demonstrate the practical side of the low volatility, two series of tests were made. The first test was made by inserting for a short time a test-tube in a 7% U. S. P. tincture to a certain mark—and an identical test-tube in a 2% iodocholeate solution—then both test-tubes were dried for five minutes in the open air; after this time the remaining iodine was removed by alcohol and titrated.

The amount of retained iodine was the same in both experiments; in other words, a 2% iodocholeate solution was found to retain as much iodine as a 7% tincture.

CHART D.—IODINE RETENTION ON GLASS.

	Sample.	Iodine Content.	Time of Exposure.	Iodine Retention.
General average of 10 experiments	U. S. P. Tincture	7%	5 min.	Equal iodine retention.
	Iodocholeate solution	2%	5 min.	

IODINE RETENTION ON SKIN (FINGER).

	Sample.	Iodine Content.	Time of Exposure.	Iodine Retention.
General average of 10 experiments	U. S. P. Tincture	7%	10 min.	Equal iodine retention.
	Iodocholeate solution	2%	10 min.	

The penetration of iodocholeate solutions was tested by the Serum Agar Cup Method, using beef agar plus 10% blood serum as medium and *Staphylococcus aureus* as test organism; a solution of iodocholeate (1.25%) showed a clear zone of 13 mm. around the cup.

It is generally recognized that organic matter decreases the germicidal effect of all halogens. Undisputedly the bile salts are organic matter, therefore, it was to be expected that the germicidal power of the iodine in the iodocholeates would also be decreased. Contrary to these expectations it was found that the germicidal effect of the iodocholeates was considerably higher when compared with the Tincture or Lugol's Solution of the same content of titrable iodine.

In the course of years of experimentation with iodocholeates, the author and others have made hundreds of bacteriological tests of these compounds; they invariably have demonstrated the fact of the superior germicidal power of the iodocholeates.

The following chart will show the bacteriological effect of the iodocholeates compared with the tincture of iodine in varying concentrations at 20° C.

CHART E.—PHENOL COEFFICIENTS (F. D. A. METHOD).

Organism: *Staphylococcus aureus* at 20° C.

Material Used.	Phenol Coefficient.	Comparative Germicidal Potency.
Iodocholeate (6.033%)	9.2	Iodocholeate
Tr. Iodine (6.033%)	5.8	58.6% higher
Iodocholeate (4.030%)	7.1	Iodocholeate
Tr. Iodine (4.030%)	4.1	73% higher
Iodocholeate (3.114%)	5.5	Iodocholeate
Tr. Iodine (3.114%)	3.2	71.9% higher
Iodocholeate (2.264%)	4.5	Iodocholeate
Tr. Iodine (2.264%)	2.3	95.6% higher
Iodocholeate (1.878%)	3.7	Iodocholeate
Tr. Iodine (1.878%)	1.9	97.1% higher

Bactericidal Test Performed upon *Bacillus subtilis* (Spores).

Material.	5 Min.	10 Min.	15 Min.
U. S. P. Tr. of Iodine 7.15%	+	+	+
Iodocholeate-sol. 2.08%	−	−	−

(+) indicates growth.
(−) indicates no growth.
F. D. A. technique used. 4-Day old culture.

Phenol Coefficient Test in the Presence of 10% Sterile Serum.

Organism: *Staphylococcus aureus* at 20° C.

Material.	Phenol Coefficient.	Germicidal Potency.
Iodocholeate (2.264%)	$\frac{40}{60} = 0.66$	Iodocholeate 164% higher
Tr. Iodine (2.264%)	$\frac{15}{60} = 0.25$	

This demonstrates that the germicidal effect of the iodocholeate solution is up to 100% and more—higher than that of the tincture. The increase in germicidal effect is in reversed proportion to the iodine content of the iodocholeate solution when compared with tincture of the same content. The lowest concentration of iodocholeate shows the highest increase in germicidal effect against tincture of the same strength.

When 10% serum is added to the dilution the germicidal action is more than 2.5 *times* higher than the one of the tincture.

The F. D. A. Method for determination of phenol coefficient was used for this test. This method has its weaknesses and not too much weight can be given to the absolute figures, but since these tests have been made under absolute identical conditions the comparative value is correct. The chart also shows the germicidal effect on spore-bearing bacteria, which are the toughest organisms. A 7% tincture of iodine fails to kill the spores in 5, 10 or 15 minutes, whereas a 2% iodocholeate solution shows definite bacteriostatic action within five minutes.

The iodocholeates possess also a very good vermicidal action. This has been established by elimination of chicken worms from live chickens. About a 2.5% solution of iodocholeate was used, and the elimination of roundworms was 100%, apparently without any ill-by-effects to the birds.

In the pharmacologic action of the iodocholeates we find a considerable difference, when compared with the Tincture of Iodine or Lugol's Solution. The most important fact is, that the iodocholeate is not harmful to the broken skin, even when kept under heavy bandages.

We know that the Tincture or Lugol's Solution or other solutions of iodine, containing even less than 2% of iodine, will cause considerable tissue damage, necrosis, eventually gangrene, when kept under bandages for a prolonged time. The injury to the tissue is in direct proportion to the iodine content of the applied solution and the time of exposure. Iodocholeate, even in higher concentration, will not cause any irritation except redness on very sensitive individuals. It was used on the skin of newly born babies without any ill after-effects.

Wounds treated with iodocholeate solutions heal much faster than those treated with Tincture of Iodine. The same is true when iodocholeate is applied on the mucous membrane. When it was given internally to experimental animals, no damage could be found on the mucosa of the stomach.

On account of the low volatility the iodocholeate has a much more prolonged action than the tincture. The technique as described by Etchells and Fabian (3) was used to demonstrate the absence of tissue damage on guinea pigs. A 2.3% Tincture of Iodine was compared with an iodocholeate solution of same iodine content. Strong necrosis appeared on the surface exposed to the tincture, but no visible effect could be noticed on the iodocholeate-exposed area.

Experiments were made on human arms, comparing Tincture of Iodine with an iodocholeate solution of same strength. In both cases a square pad of gauze was soaked with the same amount of the solutions, applied to the arm, and kept under bandages for ten hours. A picture was taken 36 hours after treatment and shows the effect of both solutions: a strong necrosis from the tincture, and practically no effect from the iodocholeate.

The effect of Tincture of Iodine and of an iodocholeate solution, when given internally by means of a stomach tube, to rabbits was demonstrated. There is practically no visible difference between the untreated stomach and the iodocholeate-treated one, but the tincture-treated stomach shows heavy injuries to the stomach linings and the stomach wall.

The comparison in the effect of a diluted Tincture of Iodine and an iodocholeate solution of the same strength was shown on rabbits' eyes. The iodocholeate-treated eye showed very little difference compared with the other eye not treated, whereas the tincture-treated eye showed heavy damage and was definitely lost.

As the iodocholeates differ in so many ways with a tincture of iodine, they offer many new opportunities for their use in pharmacy. On account of their low volatility the iodocholeates can be used for impregnation of surgical gauze, pads and tampons.

Dry powder containing iodocholeate has been prepared. The powder is odorless, of amber color, and its content of active iodine remains stable for years and is always demonstrable by starch solution. Comparative test with iodoform and thymol iodide show a very much higher germicidal effect on the iodocholeate powder. Also ointments and dental cream have been prepared.

At the present time iodine does not have the place in Surgery, Dermatology and Obstetrics, which it should have according to its merits; other very much less valuable germicides occupy its place. The drawbacks to a wider use of iodine of the type of the Tincture are their destructive action on the skin, the high volatility, its affinity for organic matter, resulting in reduction or sometimes complete destruction of the germicidal power, its painfulness in wounds and retardation of the healing process. With these factors eliminated, the possibility for the use of iodine widens considerably.

More could be said about the chemistry, pharmacology, etc., of this compound, but this will be left to another report.

The author expresses the hope that the iodocholeate will find a place where the tincture fails, and that it may become a useful contribution to the present means of fighting infection.

REFERENCES.

(1) *Ber.*, 20, 688 (1877) and *Z. physik. Chem.*, 16, 156 (1895).

(2) U. S. Food and Drug Administration Methods for Testing Antiseptics and Disinfectants, *Circular* 198, pages 13 and 15.

(3) Etchells and Fabian, *J. Ind. Hyg.*, 17, 298–300 (Nov. 1935).

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