TABLE V.—RELATION OF DEPTH OF COLOR TO MOLECULAR WEIGHT.

| Barbiturate. | Chemical Structure. | Molecular Weight. | Theoretical Reading, Standard at 20. | Actual Reading, Standard at 20. |
|---|---|---|---|---|
| Barbital | Diethyl barbituric acid | 184.112 | 20.0 | 20.0 |
| Dial | Diallyl barbituric acid | 198.112 | 21.5 | 22.2 |
| Neonal | *n*-Butyl-ethyl barbituric acid | 212.144 | 23.0 | 23.4 |
| Sandoptal | Iso-butyl allyl barbituric acid | 224.144 | 24.3 | 24.5 |
| Amytal | Isoamyl ethyl barbituric acid | 226.160 | 24.6 | 25.0 |
| Phenobarbital | Phenyl ethyl barbituric acid | 232.112 | 25.2 | 22.0 |
| Phanodorn | Cyclohexenyl ethyl barbituric acid | 236.141 | 25.7 | 24.0 |
| Ortal | *n*-Hexyl ethyl barbituric acid | 268.202 | 29.1 | 26.5 |
| Nostal | Isopropyl bromallyl barbituric acid | 289.036 | 31.4 | 32.0 |
| Pernoston | Sec-butyl bromallyl barbituric acid | 303.052 | 33.0 | 31.5 |

3. Some of the proprietary barbiturate preparations investigated did not come up to the strength stated on the label.

4. The isopropylamine test for barbiturates is sensitive enough for an approximation of the molecular weight of different barbiturates.

BIBLIOGRAPHY.

(1) Koppanyi, Murphy and Krop, *Proc. Exp. Biol. and Med.*, 31 (1933), 373.
(2) Koppanyi, Murphy and Krop, *Arch. intern. Pharm. Therap.*, 46 (1933), 76.

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# A NEW METHOD OF DETERMINING ACETYLSALICYLIC ACID IN THE PRESENCE OF MEDICINAL PRODUCTS.*

BY RICHARD M. HITCHENS.

Acetylsalicylic acid is employed so extensively in pharmaceutical preparations that its estimation in such mixtures is of considerable importance.

Many methods have been proposed for its determination. The association of Official Agricultural Chemists has carried out several systematic investigations and has proposed several methods of analysis (1). One method, applicable in the absence of acidic or basic substances, consists of titrating a cold alcoholic solution of the acetylsalicylic acid with standard alkali to a phenolphthalein end-point, thus neutralizing the free carboxyl group present in the molecule, then adding excess of standard alkali to hydrolyze the sodium acetylsalicylate to sodium salicylate and sodium acetate, and back titrating to a phenolphthalein end-point. The original titration to phenolphthalein should be exactly one-half of the total titration. Another method consists of a chloroform extraction of the acetylsalicylic acid from a water suspension, or a dry chloroform extraction of alkaline excipients is present. After removal of the chloroform the acetylsalicylic acid is determined as such if no other chloroform-soluble substances are present. Otherwise it is hydrolyzed to salicylic and acetic acids. The salicylic acid thus obtained is determined either by volumetric bromination to tribromphenol or gravimetrically by alkaline iodination to the complex $(C_6H_2I_2O)x$, structure uncertain.

In none of the methods found in the literature is acetylsalicylic acid itself separated from other organic substances and determined as such. Such a method,

* Scientific Section, A. PH. A., Washington meeting, 1934.

if feasible, would allow direct determination of and positive identification of the acetylsalicylic acid. Present methods allow only its indirect identification.

It should be possible to separate acetylsalicylic acid from many organic compounds by extracting its chloroform solution with a dilute solution of sodium bicarbonate, thus forming the water-soluble sodium salt of acetylsalicylic acid, acidifying, reëxtracting with a suitable solvent and weighing the acetylsalicylic acid after evaporating the solvent at a low temperature.

The most apparent objection to this procedure is the ease with which acetylsalicylic acid hydrolyzes to form acetic and salicylic acids, especially in neutral or alkaline solutions.

Several investigators have studied the rate of hydrolysis of acetylsalicylic acid in aqueous solution. Tsakalatoos and Horsch (2) found that complete hydrolysis occurs in aqueous solution at room temperature in about one hundred days. Germuth (3) found about 10% hydrolysis to occur at room temperature in 24 hours, using as a solvent 90% ethyl alcohol, 10% water or 90% glycerol, 10% water. Morton (4) found that hydrolysis occurs to the extent of 10% in 24 hours at room temperature in aqueous solutions containing alkali citrates or acetates.

Dott (5) found that acetylsalicylic acid could be dissolved in dilute sodium bicarbonate solution, the solution acidified, the acetylsalicylic acid extracted with a mixture of ether and chloroform, the solvent evaporated and a fairly quantitative recovery of essentially pure acetylsalicylic acid obtained. However, if the sodium bicarbonate solution were allowed to stand four hours before acidification and extraction, 4.3% hydrolysis occurred.

Experiments were accordingly started in this laboratory to determine the extent to which hydrolysis occurs when acetylsalicylic acid is extracted from chloroform solutions with a dilute sodium bicarbonate solution, the solution acidified, the acetylsalicylic acid reëxtracted and the solvent removed under reduced pressure. The free salicylic acid content of the recovered acetylsalicylic acid was determined colorimetrically by dissolving it in a little alcohol, diluting with water and matching the color produced with ferric ammonium sulphate with that produced by a known amount of salicylic acid. If the sodium bicarbonate solution was kept at 20° C. for one hour before acidification and extraction, about 0.25% hydrolysis was indicated; if at 30° C., about 0.35%. Since the salicylic acid is weighed with the acetylsalicylic acid, this would at the most cause the analysis to be 0.08% low. In practice the acetylsalicylic acid need never be in the bicarbonate solution more than 20 minutes, so the error caused by hydrolysis is negligible.

After some preliminary experiments, the following procedure was found to give quantitative recovery of acetylsalicylic acid from the chloroform solution.

About 0.3 Gm. of acetylsalicylic acid, sufficient to minimize the effect of errors in weighing, is dissolved in 80 cc. of chloroform, approximately the volume resulting from the extraction of acetylsalicylic acid from a sample of tablets. The chloroform is extracted with 25 cc. of a 2% sodium bicarbonate solution, removed to a second separator, extracted with 20 cc. of the bicarbonate solution, and discarded. The first bicarbonate extract is washed with four 10-cc. portions of chloroform, each being used to wash the second bicarbonate extract before discarding. This removes traces of chloroform-soluble substances from the bicarbonate. The bicarbonate extracts are combined and acidified with 1.5 cc. of 36% hydrochloric acid. (The bicarbonate solution should be kept at 25° C. or below and the acetylsalicylic acid should not be allowed to remain in the bicarbonate solution more than one hour.) The acidified aqueous layer is extracted

with 40, 20, 15, 10 cc. of redistilled A. R. ethyl acetate, in which acetylsalicylic acid is appreciably more soluble than in chloroform, each ethyl acetate extract being washed with 2–3 cc. of water. The ethyl acetate extracts are filtered through a plug of cotton into a small Erlenmeyer flask, carefully counterpoised against a similar flask. The ethyl acetate is evaporated under reduced pressure. This is accomplished quickly and conveniently by connecting the flask directly to a water pump and adjusting the pressure with a screw clamp so that the ethyl acetate barely boils. The process is hastened by placing the flask in a large vessel containing water at 40–45° C. In this way all ethyl acetate may be removed in 30 minutes. The flask is kept under reduced pressure for 15 minutes after the ethyl acetate has evaporated. The flask and contents are then dried to constant weight over calcium chloride. To insure rapid attainment of constant weight the counterpoise is placed in the water-bath during the evaporation.

Using this method three analyses of a U. S. P. quality acetylsalicylic acid gave 100.1%, 99.9%, 99.6% recovery. In each case the salicylic acid content of the recovered material was less than 0.15%. By double titration or by melting point, the recovered acetylsalicylic acid could not be distinguished from the starting product. The method is, therefore, quantitative, the errors being of the magnitude expected in such an analysis. The time required for an analysis is generally not over $1^1/_2$ hours.

The behavior of other compounds when subjected to this procedure was now studied to determine if acetylsalicylic acid can be separated from them by this method. The above extraction process was performed with 0.3 Gm. of acetphenetidin, caffeine, acetanilid, antipyrine, amidopyrine, phenylsalicylate, respectively, no acetylsalicylic acid being present. In no case was a weighable residue of any of the above compounds obtained, indicating that they should not interfere with the analysis for acetylsalicylic acid.

A distinct residue was obtained with phenolphthalein present. This is to be expected since chloroform is a poor solvent for this compound. If after two chloroform extractions of the bicarbonate solutions the latter are combined and extracted once with 25 cc. of ethyl acetate, the phenolphthalein is removed quantitatively.

TABLE I.

| Mixture Analyzed. | | % Recovery of |
|---|---|---|
| Acetylsalicylic Acid. | | Acetylsalicylic Acid. |
| 0.3 Gm. | 0.2 Gm. acetphenetidin | 100.1% |
| | 0.05 Gm. caffeine | 99.8 |
| 0.3 Gm. | 0.2 Gm. acetanilid | 99.8 |
| 0.3 Gm. | 0.3 Gm. antipyrine | 99.8 |
| 0.3 Gm. | 0.3 Gm. amidopyrine | 100.1 |
| 0.3 Gm. | 0.3 Gm. phenylsalicylate | 99.7 |
| 0.3 Gm. | 0.1 Gm. phenolphthalein | 99.6 |

(Sodium bicarbonate solution extracted with ethyl acetate)

With this modification, phenolphthalein does not interfere with the analysis for acetylsalicylic acid.

Mixtures of these compounds and acetylsalicylic acid were analyzed by the above procedure. The results are given in Table I. The first column gives the composition of the mixture analyzed and the second the percentage recovery of the acetylsalicylic acid. The materials used were all of U. S. P. quality. A. R. ethyl acetate was redistilled before use.

Each of these separations and determinations of acetylsalicylic acid is quantitative, the errors being within the manipulative errors of the analysis. In no case did the acetylsalicylic acid recovered contain over 0.2% free salicylic acid, indicating that the error introduced by hydrolysis of the acetylsalicylic acid is not over 0.05%.

The method can be adapted to the determination of acetylsalicylic acid in Tablets by following the A. O. A. C. procedure of preliminary extraction of the tablets with chloroform in the presence of a small amount of water or by extraction with dry chloroform if alkaline excipients are present. Using 0.3 Gm. of acetylsalicylic acid and 0.1 Gm. starch it was convenient to extract with 20, 15, 15, 10, 10, 10, 10 cc. of chloroform in the presence of 5 cc. of water. Larger volumes of water or fewer extractions with chloroform gave incomplete extraction. This method was tried on two samples of commercial tablets. The results are given in Table II.

TABLE II.

| Mixture Analyzed. | Recovery of Acetylsalicylic Acid. |
|---|---|
| 0.3 Gm. acetylsalicylic acid<br>0.1 Gm. corn starch | 100.0% |
| 0.3 Gm. acetylsalicylic acid<br>0.1 Gm. tapioca starch | 99.8% |
| Commercial Tablets: | |
| 3 1/2 grains acetylsalicylic acid | 3.41 grains per tablet |
| 2 1/2 grains acetphenetidin | 3.40 grains per tablet |
| 1/2 grain caffeine 0.7-Gm. sample | |
| 3 1/2 grains acetylsalicylic acid | |
| 2 1/2 grains acetphenetidin | 3.35 grains per tablet |
| 1/2 grain caffeine | 3.35 grains per tablet |
| 1/4 grain phenolphthalein | |

Again the known mixtures gave quantitative results. The acetylsalicylic acid content of the two samples of tablets is slightly lower than stated by the formula. However, the analyses check well, and no more acetylsalicylic acid could be detected in any of the extraction layers discarded. The materials recovered did not contain over 0.2% of free salicylic acid and were identical with acetylsalicylic acid in melting point and in double titration with alkali.

## SUMMARY.

A method is described whereby acetylsalicylic acid may be separated from many organic compounds and determined directly.

The method is rapid and accurate and allows easy identification of the acetylsalicylic acid. It is applicable in the presence of all compounds except organic acids which are relatively water insoluble, as for example, barbital, benzoic acid or salicylic acid.

## REFERENCES.

(1) Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 3rd Edition (1930).

(2) Tsakalatoos and Horsch, *Bull. soc. chim.*, 15 (1914), 743.

(3) Germuth, JOUR. A. PH. A., 20 (1931), 568.
(4) Morton, *Quant. J. Pharm. Pharmacol.*, 6 (1933), 492.
(5) Dott, *Pharm. J.*, 122 (1929), 302, 355; 124 (1930), 214.

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# ANTHELMINTICS I. THE EFFECT OF HYDROGEN PEROXIDE AND SOME OXYGENATED TERPENE HYDROCARBONS UPON *ASCARIS LUMBRICOIDES*.*

BY LEWIS W. BUTZ AND W. A. LA LANDE, JR.

The anthelmintic efficiency of oil of chenopodium in *Ascaris* infections of human beings is well established. The oil is also useful in other helminthiases (1). Its high toxicity for the host (2), (3), however, would seem to render unsafe its administration in doses sufficiently large and frequent to insure the desired anthelmintic effect. Henry and Paget (4) fractionated chenopodium oil into its constituents. These were later (5) examined separately for anthelmintic activity by Smillie and Pessoa who reported that this resided almost entirely in the ascaridole. It seems not unlikely that part of the toxicity of chenopodium oil for the human host may be due to the components other than ascaridole, *e. g.*, cymene and methyl salicylate, but apparently (2) ascaridole itself is quite toxic. It would be desirable therefore to study other substances chemically related to ascaridole with the hope of finding one with a higher therapeutic index. The work reported here represents the beginning of a proposed extended investigation having this aim in view.

The chemical structure of ascaridole (A) seems to be well established by analytic studies (6), (7), (8), although another formula has been suggested (9). It is interesting to consider whether the anthelmintic activity of this substance can be attributed to any one grouping in the molecule or whether this is due to the summation of its chemical and physical characteristics. As an approach to a solution of this question the action of hydrogen peroxide and of disuccinyl peroxide (alphozone) upon *Ascaris lumbricoides* has been studied and these substances have been found to be very toxic to the parasites. This indicates that the peroxide group, or the hydrogen peroxide or nascent oxygen arising therefrom under various conditions, has in itself pronounced anthelmintic properties. These findings logically lead to an examination of other peroxides some of which might have the desired property of low toxicity for the human host, and in addition be sufficiently stable to serve as therapeutic agents administrable *per os*.

Peroxides with these characteristics have first been sought among the terpene derivatives. It has long been known that terpenes upon exposure to air become altered and that peroxide formation is one of the processes that takes place. We have oxygenated a series of commercial and highly purified terpene hydrocarbons. In many cases products were obtained which were of the same order of toxicity to *Ascaris* as ascaridole although this toxicity probably cannot be attributed to

* Scientific Section, A. PH. A.