This derivative separated from anhydrous ether in the form of a crystalline powder which melted at 210° C.

Analysis: $CH_3CO$

$C_{27}H_{38}O_5$ Calc. for 2 acetyl groups 19.50 per cent
Found 19.68, 22.67, av. 21.17 per cent

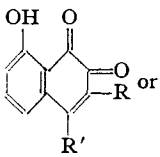
*Reaction with Bisulfite.*—Celastrol was found to be insoluble in a saturated solution of sodium bisulfite and it cannot be extracted from an ether solution with this reagent.

*Reaction with Sulfurous Acid.*—An alcohol solution of either methyl celastrol or celastrol became colorless upon the addition of sulfurous acid. The solutions became colored again upon removal of the sulfur dioxide and aeration of the mixture.

*Reaction with Orthophenylenediamine.*—Methyl celastrol was refluxed with *o*-phenylenediamine and hydrochloric acid in a hydro-alcoholic solution. The reaction mixture was diluted with water and extracted with ether. The ether solution was extracted with dilute alkali to remove any unreacted material. A very small amount of yellow needles was obtained from the ether solution. These needles melted with decomposition at 275° to 285° C.

SUMMARY

The evidence presented in Papers I and II together with subsequent investigations upon the constitution of celastrol indicates that it has the formula $C_{20}H_{32}O_8$. It is either a mono- or dialkyl substituted β- or α-naphthoquinone of one of the following tentative formulas:




where the sum of R and R′ equals $C_{12}H_{26}$.

REFERENCES

(1) O. Giswold, JOUR. A. PH. A., 28 (1939), 440; 29 (1940), 12.

(2) Hooker, S. C., *J. Am. Chem. Soc.*, 58 (1936), 1163.

(3) Fieser, L. F., *et al.*, *Ibid.*, 61 (1939), 3216.

(4) Fieser, L. F., *Ibid.*, 61 (1939), 2213; Hooker, S. C., *Ibid.*, 58 (1936), 1163.

(5) Fieser, L. F., *Ibid.*, 48 (1926), 3201; *Ibid.*, 61 (1939), 2213.

(6) *Ber.*, 66 (1933), 883.

(7) Pratt, D. S., and Perkins, G. A., *J. Am. Chem. Soc.*, 40 (1918), 228.

(8) MacCorquodale, *et al.*, *J. Biol. Chem.*, 131 (1939), 362.

---

A postage stamp in honor of the famous botanist, Karl von Linné has been issued by Sweden.

# Assay of a Variety of Vitamin $B_1$ Preparations by the Fluorophotometric Method

*By J. W. Cole, W. S. Jones and W. G. Christiansen**

Until recent years one has had to rely on biological methods for the estimation or determination of vitamin $B_1$. The disadvantages of such a situation are obvious; the cost of biological assays and the time required became factors of considerable magnitude in investigational work. The isolation, identification and synthesis of thiamin have made possible the development of rapid physiochemical methods for the estimation of vitamin $B_1$. While the status of these chemical methods is at present not such that they can replace the biological assay as a basic standard for establishing the $B_1$ potency of a product and while it is questionable as to whether that ever should be so, because the products being dealt with are intended to produce certain biological responses or effects when fed to humans (or animals), the very great usefulness of a rapid, reliable chemical method for investigational work, process control, preliminary testing so as to reduce to a minimum the amount of biological testing, stability under storage conditions, etc., is readily appreciated.

Thiamin, in both the free and phosphorylated form, is oxidized, respectively, to the free and phosphorylated thiochrome by potassium ferricyanide in alkaline solution. The free thiochrome is readily extracted by isobutanol and gives a violet-blue fluorescence in ultraviolet light. The phosphorylated thiochrome is not extracted by isobutanol. However, when the thiamin in the material to be tested exists wholly or partially in the phosphorylated form, one can by enzymatic hydrolysis, as the first step in the assay, convert the phosphorylated form into free thiamin and thus make possible the recovery of all the thiamin in the form of free thiochrome for estimation

* Control and Development Divisions, Chemical and Pharmaceutical Laboratories. E. R. Squibb & Sons, Brooklyn, N. Y.

fluorometrically. This serves as a means of learning the total thiamin content; a similar assay without enzymatic hydrolysis reveals the amount present in the free form, and the difference obviously represents thiamin present in the phosphorylated form. It is possible, therefore, to determine the character of the thiamin in the material as well as the total amount present.

fluorophotometer (Model A) manufactured by Pfaltz & Bauer, Inc., operates on the principle that an exciting beam from the light source passes through the isobutanol solution of thiochrome in a cuvette causing it to fluoresce. The fluorescent light strikes a photocell, which transforms the light into electric energy; this photoelectric current is then registered on the galvanometer.

Table I.—Thiamin Chloride and Preparations Containing It as the Only Vitamin Component

| Form of the Thiamin Chloride | No. of Samples Tested | Fluorophotometric Assay | | | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Average | Calculated Potency[a] |
| Powder | 12 | 933,000 | 1,041,000 | 999,000 | 1,000,000 γ/Gm. |
| Tablet | 6 | 1,017 | 1,170 | 1,062 | 1,000 γ/tab. |
| Tablet | 1 | 2,880 | 3,300 | 3,053 | 3,000 γ/tab. |
| Tablet | 6 | 4,840 | 5,300 | 5,010 | 5,000 γ/tab. |
| Tablet | 1 | ... | .... | 9,890 | 10,000 γ/tab. |
| Solution | 5 | 10,000 | 11,800 | 10,200 | 10,000 γ/cc. |
| Solution | 2 | 49,000 | 51,800 | 50,400 | 50,000 γ/cc. |
| Elixir | 36 | 2,720 | 3,800 | 3,250 | 3,060 γ/fl. oz. |
| Mineral oil emulsion | 2 | 1,207 | 1,224 | 1,215 | 1,150 γ/fl. oz. |

[a] Calculated on a basis of the amount of thiamin chloride in the sample tested.

By applying these principles Hennessy and Cerecedo[1] have developed a simple method or procedure for the quantitative estimation of vitamin $B_1$ fluorophotometrically. One of the important aspects of this method is that it provides a means for the assaying of complex mixtures. With thiamin alone, free from the materials with which it is associated in natural products or the materials with which it is mixed in the various multi-vitamin preparations, the estimation is reasonably straightforward, but when these other materials are present, interference is so great that the results are of little value. By contacting the solution containing the thiamin with zeolite, the "base exchange" characteristics of the latter result in the replacement by thiamin of one of the bases in the zeolite, remaining in the latter and being available for elution by a suitable liquid. This eluate contains the thiamin without the interfering substances and the assay can be made without difficulty. Hennessy and Cerecedo have published the details of the procedure and have reported on it at various scientific meetings during the last several years; it is, therefore, unnecessary to repeat them here. The instrument used, a

[1] *J. Am. Chem. Soc.*, 61, (1939) 179; A. C. S. Abstracts of Meeting, Baltimore, April 3 to 7, 1939, Division of Biological Chemistry.

For hydrolysis of any phosphorylated thiamin an enzyme preparation from beef kidney was formerly used by Hennessy; takadiastase is now used and is found satisfactory—the hydrolysis is accomplished in one and one-half hours at 40° C.

## EXPERIMENTAL

For more than a year, this laboratory has been routinely applying the fluorophotometric method of Hennessy to a wide variety of raw materials, mix-

Table II.—Natural Materials Containing the B Complex

| Material Tested | Vitamin $B_1$ in Gamma per Gm. | |
|---|---|---|
| | Fluorophotometric Assay | Bioassay |
| Yeast powder | 181 | 156 |
| Yeast powder | 177 | 165 |
| Yeast powder | 150 | 156 |
| Yeast powder | 181 | 180 |
| Wheat germ | 18.6 | 15 |
| Malted wheat germ extract | | |
| Powder | 23.8 | 21.0 |
| Powder | 20.4 | 24.9 |
| Syrup | 11.4 | 15.0 |
| Concentrated cereal extract | 162 | 186 |
| Concentrated cereal extract | 174 | 180 |
| Concentrated cereal extract | 151 | 150 |
| Concentrated cereal extract | 156 | 150 |
| Powder containing B complex | 633 | 660 |
| Powder containing B complex | 624 | 660 |
| Powder containing B complex | 602 | 660 |

Table III.—Preparations Made Using Bioassayed Natural Materials

| Preparation Tested | No. of Samples Tested | Fluorophotometric Assay Minimum | Maximum | Average | Calculated Potency[a] |
|---|---|---|---|---|---|
| *A.* Uni-vitamin products | | | | | |
| Yeast tablets | 8 | 52.5 | 68.4 | 60.6 | 60 γ/tab. |
| Yeast tablets | 33 | 47.4 | 79.2 | 58.5 | 63 γ/tab. |
| Malted wheat germ extract cpd | 17 | 6.12 | 9.3 | 6.96 | 6.4 γ/Gm. |
| *B.* Multi-vitamin products | | | | | |
| Yeast-fish liver oil tablets | 6 | 132 | 255 | 181.5 | 172.5 γ/tab. |
| Paste in capsules, Type I | 18 | 93 | 119 | 105 | 114 γ/cap. |
| Paste in capsules, Type II | 9 | 702 | 864 | 774 | 798 γ/cap. |
| Paste in capsules, Type III | 4 | 798 | 933 | 879 | 900 γ/cap. |
| Paste in capsules, Type IV | 10 | 95 | 130 | 111 | 104 γ/cap. |

[a] Calculated from the bioassay on the ingredients.

tures and finished products. For convenience of presentation the materials tested have been divided into three types and the data on each are shown in a separate table. (See Tables I, II and III.) These tabulated data require little comment. As would be expected there are variations in the results obtained by the fluorophotometric assay and there are differences between them and those obtained by bioassay or by calculation from potency data on the $B_1$ components of the products tested. However, taken as a whole findings are good, and it does appear that this fluorophotometric method is of practical value in testing for vitamin $B_1$.

One of the most important phases of this kind of work is the preparation of the sample for test. That is, of course, an easy matter when one is dealing with thiamin chloride itself or the simple forms of it such as solutions and tablets but becomes a very difficult matter when one reaches the complex multivitamin preparations such as the various pastes in capsules—obviously there are products representing practically the entire range from those easy to handle to the difficult ones. Inasmuch as each product or mixture is a problem in itself and the preparation for analysis has to be adjusted in accordance with the character and composition of the material to be tested and inasmuch as it is not the purpose of this paper to take up that phase of the work details on how to go about assaying, each type of sample will not be given. The point is that even though the complexity of the mixture does become high the assay can be run and results of the proper order are obtained.

Table III, Section *B*, relates to multi-vitamin preparations in which the fluorophotometric data were compared with figures calculated from the bioassay data as components of the mixtures instead of with the findings in bioassays on the mixture itself. For this reason and because of the difficulties associated with the assaying of these complex mixtures (see above), it is worth while to include a comparison of the fluorophotometric assay with the bioassay of the finished complex mixture. In a multivitamin paste in capsules the fluorophotometric assay showed 108 gamma of $B_1$ per capsule as compared with 99 by bioassay. In a syrupy emulsion of fish liver oil and other fats containing the *B* complex, carbohydrates, proteins, minerals and flavoring materials, the two samples gave the following results:

| | Fluorophotometric | Biological |
|---|---|---|
| *A* | 423 γ/fl. oz. | 450 γ/fl. oz. |
| *B* | 540 | 465 |

Note that when the proper method of handling mixtures like these two have been satisfactorily worked out and skill in the technique has been developed close agreement with bioassay can be had.

# A Method for the Determination of Mercury in Complex Ions*

*By John T. Read and Roger F. Maize*†

The determination of mercury in complex anions such as that occurring in potassium mercuric iodide presents a problem for which ordinary methods of analysis and available literature fail to give a satisfactory solution, the definite problem at hand being the estimation of mercury in germicidal tablets containing mercuric iodide, potassium iodide, sodium bicarbonate and an organic dye. The major difficulty consists of splitting the complex ion containing the mercury and converting the latter into a form in which it can be readily estimated.

## METHOD DEVELOPED

The details of the method as finally developed are as follows: Weigh accurately an amount of the sample estimated to contain about 0.30 Gm. mercury and transfer to a 500-cc. Kjeldahl flask.

* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.

† From the Control Laboratory of The Columbus Pharmacal Company, Columbus, Ohio.