TABLE I.—SUBSTITUTED BENZOYLINDOLIZINES

Compd.	M.p., °C.(Recrystn. Solv.)	Yield, %	Anal. Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
IV*a*	220.5 to 222.0, dec. (hot C_6H_6)	66	65.20	3.42	5.63	65.60	3.45	5.34
IV*b*	177 to 178.5 (boiling EtOH)	89	65.20	3.42	5.63	65.55	3.60	5.26
IV*c*	218 to 219.5, dec. (C_6H_6-Petroleum ether)	38	61.01	3.01	5.27	61.14	3.22	5.33
IV*d*	202 to 203.5 (C_6H_6)	64	61.01	3.01	5.27	60.61	3.18	5.48
IV*e*	245.5 to 247.0 (hot C_6H_6)	54	69.99	3.67	9.07	70.29	3.90	9.22
IV*f*	222 to 223.5	46	69.99	3.67	9.07	70.18	3.77	8.78

To test this theory, Compounds I *a*, *b*, and *c* were reacted with *p*- and *m*-nitrobenzoyl chlorides. The *p*-nitro would tend to decrease the formation of III by the conjugative mechanism (the inductive effect being negligible) and the *m*-nitro group would tend to favor the formation of III by a combination of conjugative and inductive effects (3).

These assumptions are followed with Compound I *a*, but fail in the reaction of I *b* with *m*-nitrobenzoyl chloride because the *p*-chlorophenyl substituent present in the 3 position of the indolizine does not favor the formation of II. This effect is also shown to provide a sharp decrease in the formation of IV *c*. A similar anomoly is shown between I *c* and *m*-nitrobenzoyl chloride because the steric repulsion of two proximal nitro groups is unfavorable to the formation of IV. However, the reaction of *p*-nitrobenzoyl chloride with I *c* follows the pattern set by Compound I *a*, *i.e.*, the *p*-nitro group has little effect upon the reactions in this series unless accompanied by a strong tendency to decrease the nucleophilic character of the 1 position of the indolizine nucleus.

Compounds IV *a* through *f* were prepared in the manner previously described (1); the analytical data appear in Table I.

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Quantitative Fluorometric Determination of Panthenol in Multivitamin Preparations

By RENÉ G. PANIER and JEAN A. CLOSE

A new procedure is described for the estimation of panthenol in a multivitamin preparation highly concentrated in sugar. The method involves the quantitative elimination of sugar by crystallization in the presence of ethanol, extraction of panthenol from the dry residue with chloroform, purification of the extract on ion-exchange resins, and final fluorometric determination of β-alanol after alkaline hydrolysis of the effluent. Fluorescence is developed by reaction of β-alanol with ninhydrin and *n*-butanal, for 45 minutes at 60° in the presence of carbonate buffer, pH 9.1. The fluorescent intensity measured at 465 mμ is proportional to the β-alanol concentration in the range between 0.1 and 1 mcg./ml.

DURING THE LAST 10 years, several papers have been published on the quantitative analysis of panthenol in multivitamin pharmaceutical preparations. Chemical methods have been proposed to replace the time consuming microbiological assay. It seems, however, that the problem of the determination of panthenol in highly concentrated sugar preparations has not been solved satisfactorily, and we were recently confronted with that question.

In 1949, Crockaert (1) showed that panthenol and calcium pantothenate could be determined after alkaline hydrolysis as β-alanol and β-alanine, respectively, with the use of 1,2-naphthoquinone-4-sulfonate, while Wollish and Schmall (2) proposed the dosage of pantoyl lactone as ferric hydroxamate, after acid cleaving of the panthenol.

More recently, Zappala and Simpson (3) described a colorimetric method for the determination of panthenol in multivitamin tablets containing 15% sugar. According to these authors, the method of Schmall and Wollish (4) is not applicable to the preparation highly concentrated in sucrose because of the darkening of the solution during the alkaline hydrolysis. They solved the difficulty by extraction of the powdered sample and further purification on ion-exchange resin before estimating β-alanol by chlorination and subsequent iodometry. Their method is unfortunately inapplicable to liquid multivitamin preparations, particularly to syrups with a very high sugar content (more than 50% sucrose).

Our purposes were to determine the panthenol in multivitamin preparations and to perfect a very sensitive routine method capable of answering our control and research problems.

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EXPERIMENTAL

Reagents

All the reagents were analytical grade.

Sodium hydroxide (0.5 *N*), hydrochloric acid, absolute ethanol, sucrose, and chloroform were used.

Solution A.—Composed of acetate buffer, pH 4.7, 1 *M*.

Solution B.—Composed of acetate buffer, pH 4.7, 0.5 *M*.

Solution C.—Composed of a 14 m*M* quantity of ninhydrin solution in carbonate/bicarbonate buffer, pH 9.1, 1 *M;* must be prepared freshly.

Solution D.—A standard solution of β-alanol—5 mg. pure panthenol dissolved in a few milliliters of distilled water was hydrolyzed with 2 ml. NaOH, 0.5 *N*, in a boiling water bath for 1 hour, neutralized to pH 7, diluted with distilled water to 50 ml., and then to 100 ml. with *Solution A*.

Solution E.—Composed of a freshly prepared 0.2 *M* quantity of *n*-butanal aqueous solution.

Ion-Exchange Resins.—Dowex 50X4 (200–400 mesh), Amberlite C.G. 400 (100–200 mesh) were employed. The resins are prepared as usual: repeated washings with distilled water and elimination of the fines by pouring the supernatant; alternate washings with hydrochloric acid and sodium hydroxide until clear and colorless filtrates are obtained. Finally, prepare Dowex 50 under H^+ form and Amberlite C.G. 400 under OH^- form; wash the resins with water until neutral reaction of the effluent.

Procedure

A.—Transfer a known volume of the multivitamin syrup, equivalent to 2–3 mg. panthenol in a flat-bottomed porcelain basin. A few crystals of sucrose are added; 9 volumes of chilled absolute ethanol are poured over the syrup. Allow the solution to stand overnight in the refrigerator at 4°; a slow crystallization of sucrose is obtained. Partial or total evaporation of the alcoholic solvent occurs during the night; evaporation to total dryness is achieved in a desiccator under vacuum.

B.—Thoroughly and finely powder the crystalline residue in the basin with an agate pestle; transfer the powder quantitatively in a 150-ml. glass-stoppered Erlenmeyer flask. Wash the basin carefully with a few milliliters of absolute ethanol which are added to the flask, add 80 ml. $CHCl_3$. Mechanically shake the stoppered flask for 30 minutes; then filter the chloroformic suspension on fritted glass. The flask and the sugar crystals on filter are washed out three times with 25-ml. $CHCl_3$ fractions.

Collect the main filtrate and washings together in a round bottom flask and evaporate under vacuum the clear (sometimes lightly colored) solution to dryness at low temperature (30–35°) in a rotating evaporator. The dry residue is quantitatively dissolved in 20–25 ml. distilled water in several fractions.

C.—Allow the aqueous solution to pass through a Dowex 50X4 (H^+) column—25 mm. diam. × 25 mm. height—at a rate of 6 ml./minute; immediately wash the column with 60 ml. of water. The solution and washing are collected separately. Both effluents are then passed successively through an Amberlite C.G. 400 (OH^-) column—10 mm. diam. × 100 mm. height—at a flow rate of 3 ml./minute, then collected together in a 100-ml. volumetric flask. The solution is perfectly clear and colorless; it must not give any fluorescence by U.V. irradiation.

D.—Add 5 ml. NaOH, 0.5 *N*, and heat for 1 hour in a boiling water bath. Cool the hydrolyzate and neutralize with normal hydrochloric acid; dilute to the mark with distilled water.

E.—**Fluorometric Determination.**—*Unknown.*—Pipet 0.5 ml. of the sample hydrolyzate into a test tube and add successively 0.5 ml. buffer solution, pH 4.7 (*Solution A*); 0.5 ml. butanal solution (*Solution E*); and 0.5 ml. ninhydrin solution (*Solution C*).

Standard and Blank.—Prepare standard and blank solutions as follows: 1 ml. standard hydrolyzate (*Solution D*); 0.5 ml. *n*-butanal solution (*Solution E*); and 0.5 ml. ninhydrin solution (*Solution C*). For the blank, use 1 ml. buffer solution, pH 4.7, 0.5 *M* (*Solution B*), 0.5 ml. butanal solution, and 0.5 ml. ninhydrin solution.

Mix well by swirling and allow the loosely capped tubes to stand for 45 minutes in a water bath at 60°. Cool the tubes and dilute the solutions with 18 ml. distilled water. Read the fluorescent intensity of the blank, sample, and standard solutions at an excitation wavelength of 385 mμ and fluorescence of 465 mμ with a convenient spectrophotofluorometer.

The authors use the Zeiss spectrophotofluorometer

TABLE I.—COMPARISON OF RESULTS BY FLUOROMETRIC AND MICROBIOLOGICAL METHODS

Sample Syrup[a]	Theoretical Amount of Panthenol, mg./100 ml.	Panthenol Found: Fluorometric	Panthenol Found: Microbiological[c]
1	66	64.7	60.0
2	66	66.0	64.5
3	33	31.0	...
4	66	65.7	66.0
5	66	66.3	...
6	33	32.80	...
Tablets[b]	mg./Tablet		
1	20	20.0	20.8
2	20	19.5	23.0
3	20	...	21.5
4	20	...	19.7

[a] Composition: vitamins B_1, B_2, B_6, nicotinamide, nipagine, sucrose (more than 50%). [b] Composition: vitamins B_1, B_2, B_6, B_{12}, nicotinamide, lactose (30%). [c] We are indebted to Dr. Brouwers, Microbiology Department, Union Chimique Belge, for the microbiological data. The determinations were made with *Acetobacter suboxydans* according to E. De Ritter and S. H. Rubin (6).

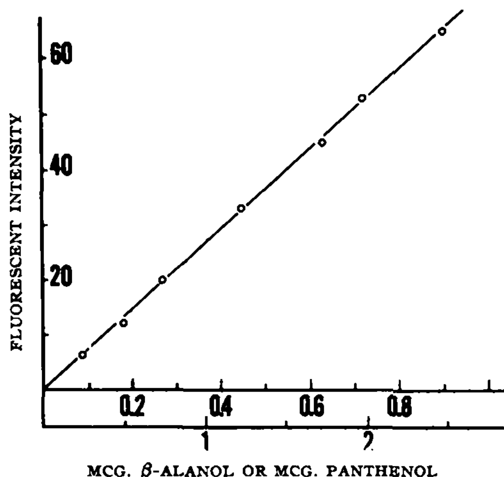

Fig. 1.—Relationship between fluorescent intensity and β-alanol concentration.

(Z.M.F.4.C.) under the following operating conditions. Calibration is made with a quinine sulfate solution (5); 1 mm. monochromator's slits are used. The sensitivity of the instrument is set so that a full scale deflection of the galvanometer spot is obtained for 3.5 mcg./ml. concentration of panthenol in reading the diluted solution, using quartz cells—1 cm. light path—for the measurements.

F.—Calculate the amount of panthenol in the unknown syrup by

$$\frac{Ps\,(Iu - Ib)}{Vu\,(Is - Ib)} \times 200 =$$

mg. panthenol in 100 ml. syrup[1]

where Iu = fluorescent intensity of the unknown, Is = fluorescent intensity of the standard. Ib = fluorescent intensity of the blank, Ps = standard panthenol content in mg./100 ml., and Vu = volume of the sample of the unknown syrup.

RESULTS AND DISCUSSION

In this laboratory the described method has given reproducible results, both with multivitamin syrups and tablets. With the latter, the first step of the procedure was neglected: the tablets were directly powdered and extracted as described in step *B*.

As shown in Table I, the mean deviation between the observed results was ±2% which indicates a good reproducibility in the range of concentration studied.

As mentioned by Zappala and Simpson (3), the recoveries of panthenol after treatment on ion-exchange resins are low because of a partial retention of the vitamin by the resins. Several assays with internal standards have shown that the mean retention in our experimental conditions was approximately 6% of the total panthenol content of the solution. A correction factor of 6% was thus introduced in the final calculation of panthenol in the syrups and tablets.

[1] Uncorrected value; see next paragraph for the correction factor.

The quantitative determination of β-alanol in the final hydrolyzate could have been made by one of the methods described in the literature (1, 3, 4). It seemed interesting to use the new fluorometric method perfected by Close, *et al.* (7), which is routinely used in our laboratory for other purposes. This method is easy, rapid, precise, and very sensitive.

As illustrated in Fig. 1, the relationship between the fluorescent intensity and the β-alanol concentration is perfectly linear in a range of concentration between 0.1 and 1 mcg./ml. of β-alanol in the diluted solution (or 0.27 and 2.7 mcg./ml. of panthenol). This range is highly convenient for the needs of the quantitative estimation of panthenol in the pharmaceutical preparations.

We believe that the method proposed can be used as a routine procedure for the panthenol determination in the pharmaceutical preparations with a high sugar content, and that it can also be the basis for the assay of the other vitamins contained in the same preparations.

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Two New Glucosides from *Cassia angustifolia* Pods

By M. L. KHORANA and M. M. SANGHAVI

Fractional precipitation and chromatographic studies have shown that *C. angustifolia* pods contain, besides sennosides A and B, glucosides of rhein and chrysophanic acid. The latter were best isolated by acidification of the aqueous extract to pH 3. Biologically, a mixture of these new anthraquinone glucosides and the bianthranol glycosides was more active than either. The possibility of the presence of traces of aloe-emodin or emodin glucoside has also been indicated.

In 1949, STOLL (1) isolated sennosides A and B from the leaves and pods of senna (*C. angustifolia* Vahl and *C. acutifolia* Delile). However, Fairbairn (2) showed that these two glycosides did not represent the full potency of the drug. Further studies of the drug with the purpose of isolating other principles, representing the residual potency of the drugs, were therefore warranted. During recent years *C. acutifolia* has been studied by Fairbairn (3) and Vickers (4), and isolation of a number of glycosides has been claimed.

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This work deals with similar studies on *C. angustifolia* pods, and evidence for the presence of two other glycosides is presented.

EXPERIMENTAL

Aqueous Extract

A coarse powder prepared from 600 Gm. of senna pods was divided into three portions. One portion, after maceration with 36 ml. of 1 *N* sodium bicarbonate and menstruum (water saturated with chloroform), was percolated with the menstruum to obtain 2 L. of the percolate. The second and third portions of the drug were similarly extracted, using first the extract from the previous batch as