mixed by inversion, and allowed to stand one hour at room temperature and for an additional 24 hours at approximately 5°. None of the solutions caused any hemolysis of the erythrocytes by the end of the 25 hours. This indicates that the saponin content is not great and the extract is not strongly hemolytic.

The powdered seeds were further investigated for the presence of alkaloids by employing the routine acidic-aqueous and alkaline-chloroform partition extraction procedure twice and then testing the aqueous solution with Mayer's reagent and, likewise, the chloroform solution on a spot plate with Erdmann's, Froehde's, and Mandelin's reagents. All tests were negative, which indicates the absence of alkaloids in cedron seeds.

SUMMARY

1. *Simaba cedron* seeds contain a weak, nonspecific, and relatively toxic anti-inflammatory substance.

2. An aqueous extract of the seeds is nonhemolytic to erythrocytes and does not contain alkaloids.

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Application of Solvent Extraction and Anion-Exchange Chromatography to the Determination of Sodium Pantothenate in Pharmaceutical Products

By T. PANALAKS

A spectrophotometric method, developed for calcium *d*-pantothenate, was demonstrated to be equally applicable to the sodium salt.

A CHEMICAL method for the determination of calcium *d*-pantothenate in pharmaceutical products was recently developed in this laboratory (1). The method was based in part on an extraction with benzyl alcohol in the presence of a salting-out agent and on an anion exchange chromatography. Since different salts were shown to affect the partition coefficient of the pantothenate in the solvent extraction, it was necessary to investigate the applicability of the method to sodium *d*-pantothenate which is used in some pharmaceutical products. The possibility of using the calcium salt as a reference standard in the determination of the sodium form was also studied by comparing the relative absorbance produced by the reaction product of the two salts.

EXPERIMENTAL

Preparation of Sodium Pantothenate.—A 5.0-Gm. quantity of calcium *d*-pantothenate was dissolved in a minimum volume of distilled water and 21.5 ml. of a 5% solution of sodium carbonate was added with stirring. The solution was let stand overnight in a refrigerator, then filtered on a No. 1 Whatman. The precipitate was washed with about 350 ml. distilled water. The combined filtrate and washings was adjusted to pH 6.0, and made up to 500 ml. with distilled water.

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Recovery Test.—Amounts of 1.235, 2.470, and 4.940 ml. of the prepared solution were each added to 25 ml. of a liquid multivitamin preparation. These amounts were equivalent to 0.5, 1.0, and 2.0 mg. of sodium *d*-pantothenate per ml. of the liquid sample. The determinations were made in duplicate on aliquots of 10 ml. of the solutions after being diluted to a 100 ml. with distilled water. Calcium *d*-pantothenate was the reference standard and a factor of 1.0124 was used for the conversion of calcium *d*-pantothenate to its equivalent of the sodium salt.

RESULTS AND DISCUSSION

To compare the behavior of the two salts, 5 mg. of the calcium salt and an equivalent weight of the prepared sodium salt were subjected to the procedure (1). The assays were conducted in three replicates on different days. The results, presented in Table I, indicated that although there was significant variation between assays for the sodium salt there was no significant difference on the average between the absorbance of the reaction products of the two salts. No apparent destruction of the pantothenate radical due to hydrolysis was observed. Since it was shown previously that the presence of amino acids did not significantly affect the final absorbance readings (1), and that an equal response of both forms of the pantothenate was obtained in this experiment, it was concluded that β-alanine was not formed before the pantothenate was subjected to the complete analytical procedure.

The results of the recovery test of sodium *d*-

TABLE I.—RELATIVE ABSORBANCE[a] OF REACTION PRODUCTS OF CALCIUM AND SODIUM PANTOTHENATE

Determination No.	Assay 1 CaPan	Assay 1 NaPan Equivalent	Assay 2 CaPan	Assay 2 NaPan Equivalent	Assay 3 CaPan	Assay 3 NaPan Equivalent
1	145	129	149	141	145	156
2	144	132	147	148	149	152
3	138	131	144	141	143	146
4	133	137	141	153	163	150
5	146	125	154	137	138	160
Mean	141.2	130.8	147.0	144.0	147.6	152.8
Std. Error	2.5	2.0	2.2	2.9	4.2	2.4

[a] Absorbance × 100 per mg.

TABLE II.—RECOVERY OF SODIUM *d*-PANTOTHENATE ADDED TO A COMPLEX VITAMIN PREPARATION

Sodium *d*-Pantothenate, mg./ml. Added	Found	Recovered
0	0.25	...
0.50	0.83	0.58
1.00	1.38	1.13
2.00	2.20	1.95

pantothenate added to a liquid elixir containing a mixture of the B vitamins and yeast extracts, as shown in Table II, indicated a recovery of 97–116%.

Samples of typical multivitamin preparations containing sodium *d*-pantothenate were analyzed, using calcium *d*-pantothenate as a reference standard. Three of these samples were in a lyophilized form. The results, shown in Table III, indicated a reasonable mean overage of 20% of the declared potency.

It may be concluded that the spectrophotometric method developed for calcium *d*-pantothenate is equally applicable to the determination of its sodium salt in pharmaceutical products, and that calcium *d*-pantothenate may be used as a reference standard.

TABLE III.—SODIUM *d*-PANTOTHENATE CONTENT OF MULTIVITAMIN PREPARATIONS

Sample	Mg. per ml. or Vial — Label Claim	Found
1. Injectable, lyophilized, B vitamins,[a] C, gentisic acid ethanolamide	20	22.7
2. Injectable, lyophilized, B vitamins,[a] C	5	7.13
3. Injectable, lyophilized, B vitamins,[a] C	5	5.20
4. Liquid drops, B vitamins,[a] A, C, D	8.33	10.2

[a] Including thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, niacinamide, vitamin B_{12}, and sodium *d*-pantothenate.

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Thin-Layer Chromatographic Identity Test for Steroids in Sesame Oil Preparations

By B. P. KORZUN and S. BRODY

A thin-layer chromatographic method is described as a rapid identity test for methandrostenolone,[1] estradiol dipropionate,[2] testosterone propionate,[3] desoxycorticosterone acetate,[4] and aldosterone acetate in sesame oil preparations. Preliminary extractions are not necessary to obtain desired results.

IT WAS desirable to obtain a rapid method for identifying steroids and decomposition products in sesame oil preparations. Attempts to apply paper chromatography failed because the sesame oil interfered, and the low concentration of steroid present did not give discernible spots with the usual reagents. Thin-layer chromatography, a technique described by Stahl (1, 2), using silica gel G as the adsorbent gave good results. The steroid is well separated from the oil and is easily detected with a modified Le Rosen reagent (3). The time required to complete the test is one to two hours.

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[1] Marketed as Dianabol by CIBA Pharmaceutical Co.
[2] Marketed as Ovocylin by CIBA Pharmaceutical Co.
[3] Marketed as Perandren by CIBA Pharmaceutical Co.
[4] Marketed as Percorten by CIBA Pharmaceutical Co.

EXPERIMENTAL

Apparatus.—The apparatus for coating the glass plates and other accessories is commercially available from Research Specialties Co., Richmond, Calif.; Arthur H. Thomas Co., Philadelphia, Pa.; and Desaga, Heidelberg, Germany. The adsorbent used is silica gel G.[5]

Preparation of Plates.—A slurry of 30 Gm. of silica gel G and 60 ml. of water is prepared and poured into applicator and spread over the plates.

[5] Manufactured according to specifications of E. Stahl by Merck, A. G., Darmstadt, Germany, and available through Research Specialties Co. and Terra Chemicals, Inc., New York.