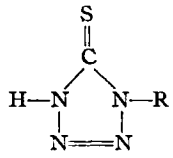
TABLE I.—1-SUBSTITUTED-TETRAZOLINE-5-THIONES



R	M.p. °C	Yield,[a] %	Nitrogen, % Calcd.	Nitrogen, % Obs.[b]	Infrared Spectra[c, e, f] —N—C=S	C=S	Cyclic —N—N=N	Tetrazole Skeletal Bands			
Methyl	125–26	52	48.2	48.5	1510(m)	1350(m)	1300(m)		1066(w)	1042(s)	
Ethyl	50	37	...	...	1500(w)		1275(s)	1088(s)		1045(s)	983(m)
n-Propyl	77–78	24	38.8	40.0	1510(s)	1350(s)	1290(m)	1112(m)		1050(s)	995(s)
iso-Propyl[d]	89–90	34	38.8	38.7							
Allyl	153	22	39.5	39.5	1500(s)	1350(m)	1304(m)		1075(m)	1053(s)	990(s)
iso-Butyl[d]	64–65	25	35.4	35.4							
t-Butyl[d]	98–99	29	35.4	35.4							
Benzyl	148	87	31.1	31.9	1495(m)		1290(w)	1090(w)		1053(m)	

a Average yield based on two runs per compound. *b* Weiler and Strauss, Microanalytical laboratory, Oxford, England (average of two runs). *c* Dr. Paul R. Caudill, College of Agriculture, University of Kentucky, Lexington. *d* Not subjected to infrared studies. *e* Perkin Elmer 21 instrument used; Nujol phase; filter out; NaCl prism; 927 program; speed 1 μ/min.: response 1; suppression 0. *f* Key: (s) = strong band; (m) = medium band; (w) = weak band.

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Determination of Ergotamine and Ergotaminine in Pharmaceutical Preparations

By THOMAS G. ALEXANDER

Ergotamine and its diastereoisomer, ergotaminine, are separated from each other and from other ingredients by column chromatography. The isolated fractions are then assayed by the classical van Urk method. Identity and purity of the fractions are confirmed by paper chromatography. A number of commercial products were analyzed successfully.

ERGOTAMINE in solution forms an equilibrium mixture of the diastereoisomers, ergotamine, and ergotaminine. This study was undertaken to develop a practical quantitative method for the assay of both ergotamine and ergotaminine in injection solutions. Of the different techniques that have been described for this purpose, column chromatography appeared to be the best suited. Larger amounts of alkaloid can be recovered more efficiently on a column than with methods involving the extraction of spots from paper or thin-layer chromatograms (1–3). Yet large samples are not required as with the polarimetric methods (4). Also a column chromatographic technique does not require the use of as much time and specialized glassware as would one involving countercurrent extraction (5).

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The method presented involves partition chromatography using a column prepared by adsorbing 1:4 citric acid solution on siliceous earth. This column extracts ergotamine from a chloroform solution containing both ergotamine and ergotaminine. The latter passes through in the effluent and is assayed. Ergotamine is recovered by chloroform extraction of the extruded column. This procedure was submitted to the Committee on Revision of the "United States Pharmacopeia" and it has been incorporated into the Monograph for Ergotamine Tartrate Injection. The procedural details are contained in this revised monograph (6).

To analyze ergotamine salts and simple tablet mixtures, weighed portions are dissolved in or triturated with 1:100 tartaric acid solution; the sample is then analyzed in the manner described in the revised monograph for injections.

EXPERIMENTAL AND DISCUSSION

In following the methods described by Berg (7) and van de Langerijt (8) involving the use of benzene as eluent, both ergotamine and ergotaminine rapidly epimerized and deteriorated. Carless (9)

TABLE I.—ASSAYS OF SIMULATED INJECTION SOLUTIONS

Sample	Isomer[a]	mg. Added (as tartrates)	mg. Found	Levo Added, %	Levo Calcd., %	Recovery of Both Isomers, %
A	Levo	5.11	5.14	100	99.0	101.6
	Dextro	0	0.05	...	...	...
B	Levo	4.09	3.94	81.3	81.6	96.0
	Dextro	0.94	0.89	...	...	...
C	Levo	2.66	2.56	54.1	52.6	99.0
	Dextro	2.26	2.31	...	...	...
D	Levo	0	0.14	0	3.0	100.6
	Dextro	4.69	4.58	...	...	...

[a] The levorotatory alkaloid is ergotamine. The dextrorotatory alkaloid is ergotaminine.

TABLE II.—ASSAY RESULTS

Sample	Ingredients Other Than Ergotamine	Per Cent of Declared, or of Amount Added — Analyst I *l*	Analyst I *d*	Analyst II *l*	Analyst II *d*	Analyst III *l*	Analyst III *d*
Simulated Injection	None	67.5	33.4	68.0	32.3	...	...
		66.9	33.3	68.9	32.1	...	...
Commercial Tablets	Phenobarbital Belladona salts	74.0[a]	10.6[a]	...	...	75.2[a]	13.0[a]
		71.8	15.3	...	...	...	...
Commercial Tablets	Phenobarbital Caffeine	69.3[a]	19.0[a]	...	...	71.1[a]	19.8[a]
		69.5	20.5	...	...	...	...
Ergotamine Tartrate	...	75.9	14.2[b]	...	...	...	...
		77.0	14.1[b]	...	...	...	...
Commercial Suppositories	Caffeine	61.5	26.5	(each line of different batch)			
	Phenobarbital sodium	65.0	22.0	...	...	...	...
Cocoa-butter	Belladona salt	18.0	52.0	...	...	...	...
Simulated Suppositories Wecobee NC 6112[c]	Caffeine Acetophenetidin Belladona salt	94.8	2.4	...	...	...	...
				...	...	...	...

[a] These results were obtained on individual whole tablets. [b] This ergotamine tartrate was unusually impure. The fact that only about 90% of the sample used was received would indicate that either part of the material was retained by the first column or if it came through in either the ergotamine or ergotaminine fractions, it did not respond to the color reaction. In either case, it could not be closely related to ergotamine. Infrared data indicated that the material in the *dextro* fraction was not ergotamine but might have been of the ergotoxine group or one of the "aci-ergot" alkaloids (13). [c] The author is grateful to E. F. Drew and Co. for supplying this hydrogenated vegetable oil suppository base.

recovered only 80% of the ergotamine in pyridine-ether effluents from columns of cellulose treated with McIlvaine citrate-phosphate buffer. In attempting to improve upon Carless' method, it was learned that both phosphate salts and pyridine have detrimental effects on ergotamine.

The sharpness with which citric acid columns will resolve mixtures of ergotamine and ergotaminine in chloroform varies with the strength of the acid. Columns prepared with strong 1:3 citric acid solution will sharply separate the diastereoisomers, but an excessive volume of effluent is required to elute completely the ergotaminine. With columns using weaker acid solution, 1:5, the ergotamine and ergotaminine fractions overlap. The column developed (9 Gm. siliceous earth plus 7 ml. of 1:4 citric acid solution) retains ergotamine, while about 97.5% of the ergotaminine appeared in the first 100 ml. and another 1 or 1.5% was obtained in the next 100 ml. Recoveries of 97 or 98% of the ergotamine were obtained by extraction of the extruded and alkalized column. The completeness of these separations was also checked by paper chromatography, using a modified version (10) of Stoll and Rüegger's reversed-phased method (11). After aliquots of the two chloroform extracts were removed for assay, the remaining portions were evaporated to a concentration suitable for spotting on paper chromatograms. Ergotamine was not detected in the ergotaminine fractions tested. Occasionally, trace amounts of ergotaminine were detected in the ergotamine fraction.

To check further the validity of the method, four simulated injection solutions were prepared, using ergotamine bitartrate freshly recrystallized from methanol and ergotaminine prepared by Stoll's method (12). These solutions were then assayed promptly. The results are presented in Table I.

ADAPTATION TO COMPLEX MIXTURES

For the analysis of ergotamine tartrate tablets and suppositories containing caffeine, acetophenetidin, barbiturates, and belladona alkaloids[1] it is necessary that the ergot alkaloids first be separated from the other ingredients prior to the isolation of ergotamine from ergotaminine. For this purpose, a previously published method was found to be suitable (10), whereby ergotamine and ergotaminine are obtained together in chloroform solution isolated from the other nitrogenous bases. This chloroform solution is then passed onto the citric acid column and the analysis continued as with the simple formulations.

RESULTS

Several mixtures were prepared to simulate commercial products. These and several commercial preparations were analyzed. The assay results are

[1] This method is not applicable to preparations containing cyclizine.

presented in Table II. Identity and purity checks by paper chromatography showed that (except as noted) no ergot alkaloids other than ergotamine or ergotaminine were detected in the products. In some older samples with low assay values, brown spots appeared at $R_f = 0$ on the paper chromatogram of the ergotaminine fraction. These probably represent ergotamine that has oxidized or otherwise deteriorated.

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Determination of Zinc in Insulin Preparations

By BENNIE ZAK and JERRY S. COHEN

A description of an accurate absorptiometric technique for the determination of zinc in insulin is presented. It involves differential demasking of zinc in the presence of contaminating interferences such as copper and iron, followed by a reaction with the color reagent, zincon. It appears to be a simple yet useful process for quality control.

AN IMPORTANT analytical phase for the pharmaceutical industry involves quality control of products (1). The determination of zinc in insulin preparations for quality control purposes is an assay which presents some difficulty. Included among the procedural techniques for this element are processes employing spectrophotometry (2, 3), polarography (4, 5), and chelatometric titrations (6, 7). The first two types of procedure involve selective dithizone extractions followed by colorimetric measurement and retrograde extraction followed by polarographic measurement. The titrimetric procedure with a complexone titrant becomes more difficult and subjective as the concentration of zinc decreases. One milliliter of 0.001 *N* ethylenediamine tetraacetic acid corresponds to 65.4 mcg. of zinc (8); some insulin preparations, such as the unmodified variety, contain only 6 mcg. of zinc per ml. A visual titration involving a two-color indicator, such as Eriochrome black T, is subject to serious shortcomings when the titrant becomes very dilute, and the indicator color-change represents a slow transition from one form to the other (9).

The procedure for insulin zinc analyses to be discussed here involves an absorptiometric technique without extraction. It is capable of achieving an accurate determination for zinc yielding high absorbance data on samples which are never greater than 1 ml., even for those preparations containing the smallest amounts of zinc (10, 11). In the case of zinc-insulin solutions, the required amount of material is a micro quantity.

EXPERIMENTAL

Reagents.—Borate buffer, pH 9.0: dissolve 31 Gm. of boric acid in metal-free distilled water, add 53 ml. of 4 *N* NaOH, and dilute the solution to a liter with any necessary adjustment of pH. Zincon color reagent: dissolve 130 mg. of zincon in 2 ml. of the detergent, acationox, and dilute the solution to 100 ml. with metal-free distilled water. Stock standard zinc solution (100 mg./L.): dissolve 100 mg. of zinc in a minimum amount of HCl and dilute the solution to a liter with metal-free distilled water. This stock is diluted with water so that 0–18 mcg. per aliquot was used as a working standard.

Procedure.—An aliquot containing approximately 6–18 mcg. of zinc was pipeted into a centrifuge tube and diluted to 3.0 ml. with metal-free distilled water. This aliquot ranged from 0.05 ml. for an 80-unit globin-zinc-insulin preparation to 1.0 ml. for a 40-unit unmodified insulin preparation. To the diluted insulin solution, 1.5 ml. of 1 *N* HCl was added to liberate the zinc from its protein binding site. Ten per cent trichloracetic acid (1.5 ml.) was then mixed in to precipitate the proteins. Trichloracetic acid may be capable of performing both functions, but the stronger acid ensures that the zinc will become unbound. After a 5-minute stand, the mixture was centrifuged, and a 4.0-ml. aliquot was transferred to a Coleman 19 mm. O.D.

TABLE I.—PRECISION OF RECOVERY OF ABSOLUTE QUANTITIES OF ZINC

3.0 Mcg.	5.0 Mcg.	7.0 Mcg.	9.0 Mcg.	12.0 Mcg.
3.0	4.9	6.8	8.9	12.3
3.0	5.0	6.8	8.8	11.9
2.9	4.8	6.9	9.0	11.9
2.9	5.0	6.9	9.0	12.3
3.1	5.0	7.1	9.0	12.3
3.1	4.9	6.8	8.9	12.3
3.0	5.0	6.9	9.2	11.9
3.1	4.9	7.1	8.8	12.0
3.0	5.0	7.0	9.0	11.9
3.1	4.8	6.9	9.2	12.0

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