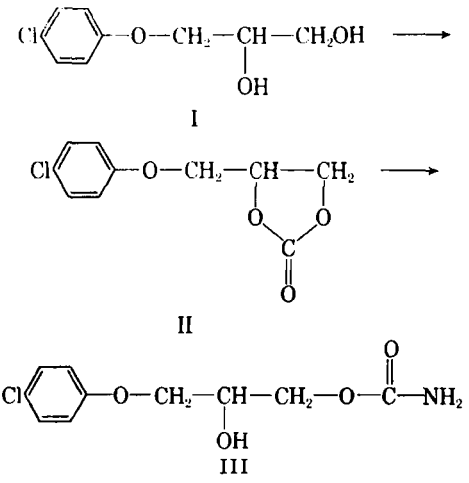
Determination of Residual Chlorphenesin in Chlorphenesin-1-carbamate

By ARLINGTON A. FORIST and RAY W. JUDY

A rapid, sensitive, and specific method for the determination of residual chlorphenesin in chlorphenesin-1-carbamate has been developed. The procedure involves oxidation with periodic acid in 50 per cent ethanol followed by spectrophotometric determination of the formaldehyde produced. Neither chlorphenesin-1-carbamate nor the possible trace by-products interfere. Over the range 2 to 5 per cent chlorphenesin, a mean recovery ± standard deviation of 99.6 ± 2.1 per cent has been found.

CHLORPHENESIN-1-CARBAMATE[1] (III), a new muscle relaxant (1), is prepared from chlorphenesin (I) *via* the cyclic carbonate (II). The isomeric chlorphenesin-2-carbamate is also formed in the



I

II

III

reaction. Likely by-products in the bulk drug are therefore this isomer, the cyclic carbonate, and chlorphenesin. The isomer content may be determined by nuclear magnetic resonance spectroscopy (2) and residual carbonate is readily determined by a quantitative infrared assay based on the carbonyl absorption at 1800 cm^{-1}. The present report describes a simple spectrophotometric procedure for the determination of residual chlorphenesin.

EXPERIMENTAL

Reagents.—The following reagents were used: periodic acid, 0.1 *M;* sodium arsenite, 1 *M*— a mixture of 25 Gm. of arsenious oxide and 11.3 Gm. of sodium hydroxide dissolved in water and diluted to 250 ml.; sulfuric acid, 2 *N;* sulfuric acid, 2:1 (v/v), prepared by mixing 2 vol. of concentrated sulfuric acid, slowly with cooling, with 1 vol. of water; chromotropic acid reagent—a 0.5-Gm. sample of recrystallized chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) dissolved in 50 ml. of water and slowly diluted, with mixing and cooling, to 250 ml. with the 2:1 sulfuric acid.

Apparatus.—Glass-stoppered tubes prepared by sealing the ends of 24/40 outside ground joints, a boiling water bath, and a Beckman model B spectrophotometer equipped with 1-cm. cells were employed.

Received January 20, 1964, from the Biochemical Research Division, The Upjohn Co., Kalamazoo, Mich.
Accepted for publication February 12, 1964.

[1] Trademarked as Maolate by the Upjohn Co.

Procedure.—Ten to twelve milligrams of sample, containing no more than 10% residual chlolphenesin, are accurately weighed into a 10-ml. volumetric flask and dissolved in 1 ml. of 95% ethanol. One milliliter of 0.1 *M* periodic acid is added, the solution is mixed, and oxidation is allowed to proceed for 15 minutes. One milliliter of 1 *M* sodium arsenite is then added, followed by 1 ml. of 2 *N* sulfuric acid. After at least 10 minutes, the resulting solution is diluted to 10 ml. with water and mixed thoroughly. One milliliter of this solution is then transferred to a glass-stoppered test tube, followed by 10 ml. of the chromotropic acid reagent. The solution is mixed thoroughly, and the stoppered tube is immersed in a boiling water bath for 30 minutes. The sample is then removed, cooled, and its absorbance determined at 570 mμ *versus* a reagent blank similarly treated. Chlorphenesin content is calculated from a linear standard curve.

RESULTS AND DISCUSSION

The present procedure is based on the production of formaldehyde from chlorphenesin by oxidation with periodic acid. The method is a modification of the procedure of Lambert and Neish (3) with the oxidation conducted in 50% ethanol to achieve complete solution of the samples involved. Results in Table I show that formaldehyde production from chlorphenesin is complete within 15 minutes at room temperature, and the resulting formaldehyde is stable for at least 2 hours in the reaction mixture.

Success of the present approach requires that chlorphenesin-1-carbamate, its isomer, and the cyclic carbonate be stable to hydrolysis and oxidation under the conditions employed. Results in Table I show that the two carbamates and the cyclic carbonate are completely stable for at least 45 minutes in the oxidation medium. Similar stability of carbamates in periodic acid was observed in earlier studies with the antibiotic novobiocin (4). Therefore, the present method is specific for chlorphenesin in the system under study.

TABLE I.—EFFECT OF OXIDATION TIME ON FORMALDEHYDE PRODUCTION

Oxidation Time, Min.	A_{570}			
	I[a]	II[b]	III[c]	IV[d]
15	0.341	0	0	0
30	0.340	0	0	0
45	0.341	0	0	0
60	0.347	...	...	...
90	0.346	...	...	...
120	0.346	...	...	...

[a] Chlorphenesin, 0.5-mg. sample. [b] Cyclic carbonate, 3.0-mg. sample. [c] Chlorphenesin-1-carbamate, 10-mg. sample. [d] Chlorphenesin-2-carbamate, 1.56-mg. sample.

TABLE II.—ABSORBANCE AS A FUNCTION OF CHLORPHENESIN SAMPLE SIZE

Sample, mg.	A_{570}	A_{570}/mg.[a]
0.10	0.062	0.620
0.20	0.139	0.695
0.30	0.194	0.647
0.40	0.260	0.650
0.50	0.323	0.646

[a] Mean ± standard deviation = 0.652 ± 0.027.

Absorbance at 570 mμ follows Beer's law over the range 0.10 to 0.50 mg. of chlorphenesin per sample (Table II). A mean absorbance per milligram ± standard deviation of 0.652 ± 0.027 has been observed, indicating adequate precision.

Application of the method to typical synthetic mixtures of chlorphenesin and chlorphenesin-1-carbamate is shown in Table III. Over the range 2 to 5% chlorphenesin, a mean recovery ± standard deviation of 99.6 ± 2.1% has been found.

The method described is rapid, sensitive, and specific for chlorphenesin in the system studied. The range 1 to 10% chlorphenesin is covered by the method as outlined; different ranges may be accommodated by suitable changes in sample size.

TABLE III.—DETERMINATION OF CHLORPHENESIN IN SYNTHETIC MIXTURES WITH CHLORPHENESIN-1-CARBAMATE

Chlorphenesin, %		
Taken	Found	Recovery, %[a]
1.96	1.95	99.5
2.91	2.98	102.4
3.85	3.81	99.0
4.76	4.63	97.3

[a] Mean ± standard deviation = 99.6 ± 2.1.

REFERENCES

(1) Matthews, R. J., DaVanzo, J. P., Collins, R. J., and VanderBrook, M. J., *Arch. Intern. Pharmacodyn.*, 143, 574(1963).
(2) Slomp, G., Baker, R. H., Jr., and MacKellar, F. A., *Anal. Chem.*, 36, 375(1964).
(3) Lambert, M., and Neish, A. C., *Can. J. Research*, 28B, 83(1950).
(4) Forist, A. A., Theal, S., and Struck, W. A., *Anal. Chem.*, 31, 100(1959).

Interaction of Low Molecular Weight Polyethylene Glycols with Sorbitol Solution

By JOSEPH ROBINSON, MILO GIBALDI, NORMAN D. WEINER, and JOSEPH L. KANIG

A single-phase solid material is produced when liquid polyethylene glycols and sorbitol solution U. S. P. are mixed. Various factors which influence this interaction have been explored. Temperature, components, component ratio, and soluble salts affect the rate of the interaction but do not affect the product itself. A nonspecific mechanism of dehydration of the sorbitol solution is apparently involved.

OBSERVATIONS MADE in our laboratories had shown that a waxlike solid material was produced when two widely used pharmaceutical liquids, polyethylene glycol 400 U.S.P. and sorbitol solution U.S.P., were intimately mixed. This solid substance was found to be soluble in water in all proportions and to melt in a range of 35 to 40°.

A survey of the literature indicated that this phenomenon had never been reported. This fact, in addition to the potential pharmaceutical applications of a material with such solubility and fusion characteristics, prompted further investigation.

Both sorbitol and polyethylene glycol (PEG) are ordinarily chemically inert. The oxyethylene moiety of PEG, however, is known to associate with compounds that possess an "active" hydrogen atom. This type of hydrogen bonding occurs between PEG and water, alcohol, and phenols (1). The strong tendency of PEG to form hydrogen bonds has been shown to be of pharmaceutical importance in such instances as the inactivation of salicylates (1) and a number of phenolic preservatives (2). An illustration of the extent of this association is the strong solvation of the polymer in water, which results in its complete solubilization.

Received February 24, 1964, from the College of Pharmacy, Columbia University, New York, N. Y.
Accepted for publication March 19, 1964.

A study was initiated to investigate certain variables which would provide an insight into the possible mechanism of this liquid-liquid interaction.

EXPERIMENTAL RESULTS

Procedure.—Sorbitol solution U.S.P. [containing 70% (w/w) D-sorbitol in water] and the various low molecular weight polyethylene glycols used in this investigation were maintained at 25° for at least 1 hour prior to mixing. Mixing of the components was accomplished in a 600-ml. beaker immersed in a water bath at 25 ± 0.2°. The total weight of the mixture used in all experiments was 100 Gm.

The addition of the liquid PEG to sorbitol solution results in two distinct liquid phases. Gentle stirring immediately produces a hazy cloud within the mixture. Upon continued stirring, the viscosity of the mixture gradually increases until a solid material, with no apparent liquid phase, is produced. It was noted that an increase in the rate of stirring greatly decreased the time required to solidify the mixture. Intermittent manual stirring required about 2 hours to form the solid, whereas high-speed agitation with a model F Lightnin mixer produced equivalent results in considerably shorter periods of time. The transition from the fluid to the solid state was clearly defined and gave rise to an end