at equimolar ratios of tetracycline to cation[5] (3). Therefore, even partial complexing can have important effects on both *in vivo* and *in vitro* tetracycline interactions.

REFERENCES

(1) J. T. Doluisio and A. N. Martin, *J. Med. Chem.*, **6**, 16 (1963).
(2) A. Albert and C. W. Rees, *Nature*, **177**, 433 (1956).
(3) T. Chin and J. L. Lach, *Am. J. Hosp. Pharm.*, **32**, 625 (1975).
(4) D. R. Crow and J. V. Westwood, "Polarography," Methuen, London, England, 1968, pp. 63–79.
(5) J. J. Lingane, *Chem. Rev.*, **29**, 1 (1941).
(6) L. Meites, "Polarographic Techniques," 2nd ed., Interscience, New York, N.Y., 1965, p. 571 ff.
(7) "Physicians' Desk Reference," Medical Economics Co., Oradell, N.J., 1978, pp. 956, 1285.
(8) D. A. Skoog and D. M. West, "Fundamentals of Analytical Chemistry," 3rd ed., Holt, Rinehart and Winston, New York, N.Y., 1976, p. 469.
(9) R. H. Harris, in "Handbook of Drug Interactions," 2nd ed., E. A. Hartshorn, Ed., Drug Intelligence Publications, Hamilton, Ill., 1973, p. 158.

[5] A. Cutie and T. Jochsberger, unpublished results.

ACKNOWLEDGMENTS

Presented at the APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1978.

Photolytic Decomposition of Indapamide

REG DAVIS *x, CLIFFORD H. J. WELLS *, and ALBERT R. TAYLOR ‡

Received December 20, 1978, from the **School of Chemical and Physical Sciences, Kingston Polytechnic, Kingston upon Thames KT1 2EE, England,* and ‡*Servier Laboratories, Ltd., Greenford, Middlesex UB6 7PW, England.* Accepted for publication February 7, 1979.

Abstract □ Photolytic decomposition of indapamide (I) in nitrogen-flushed methanol yields 3-sulfamoyl-4-chlorobenzamide (II), 2-methylindoline (III), semicarbazide (IV), and 1-(*N*-formamido)-2-methylindoline (V); in oxygen-flushed methanol, II–V, 1-aminocarboxymethyl-2-methylindoline (VI), 3-sulfamoyl-4-chlorobenzoic acid (VII), methyl-3-sulfamoyl-4-chlorobenzoate (VIII), and 2-(*N*-acetamido)-benzoic acid (IX) are formed. A comparison is made with thermal decomposition of I.

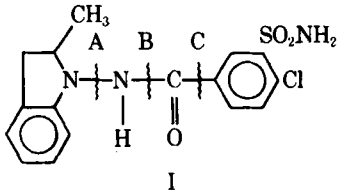
Keyphrases □ Indapamide—photolytic decomposition, thermal decomposition, decomposition products identified □ Photolysis—indapamide, products identified □ Thermolysis—indapamide, products identified □ Diuretics—indapamide, photolytic and thermal decomposition, products identified

There has recently been a growth in drug photosensitization studies. Numerous drugs have photosensitizing properties (1), and these properties appear to show a correlation with *in vivo* photosensitivity. In particular, the saluretic furosemide exhibits a high affinity for oxygen under UV irradiation, and this affinity may be related to the reported skin rash reaction among patients taking the drug (2).

As part of detailed studies of indapamide [*N*-(3-sulfamoyl-4-chlorobenzamido)-2-methylindole] (I), a compound with prolonged saluretic action (3), a study of the photolytic decomposition of this compound was conducted. In contrast to earlier studies (1), the products of *in vitro* photolytic decomposition were identified prior to *in vivo* experiments.

EXPERIMENTAL[1]

Indapamide (I) was supplied as a pure compound[2]. 2-Methylindoline (III), semicarbazide (IV), and 2-(*N*-acetamido)benzoic acid (IX) were supplied as pure reference compounds[3].



I

Photolytic decompositions were performed on 2.7×10^{-3} *M* I solutions in nitrogen- or oxygen-flushed methanol. The reactant solutions were contained in the inner well of a cylindrical quartz reaction vessel, which was mounted vertically before a 1000-w Hanovia medium-pressure mercury lamp. A filter solution of saturated aqueous copper sulfate was pumped through the annular space (4-mm i.d.) surrounding the inner well of the reaction vessel and also was pumped through a heat-exchanger to maintain the reactant solution at 293°K. The filter solution had a short wavelength cutoff at 300 nm. After photolysis for 12 hr, the solution was evaporated to dryness under reduced pressure. The residue was dissolved in a small volume of redistilled methanol prior to spotting on TLC plates.

Thermal decompositions were performed by heating samples in an automatic thermo-recording balance[4]. A 1-g sample of I was heated either at a constant rate of 5°K/min from ambient temperature to 453°K until the weight loss rate stabilized or at 453°K for 12 hr while the sample underwent a regular and continuous weight loss. The solids were dissolved in redistilled methanol prior to spotting on TLC plates.

Preparative TLC was performed using 20 × 20-cm plates coated with a 1-mm layer of silica gel[5]. Two solvent systems were used for plate development: 1, 100% dimethyl carbonate; and 2, the top layer obtained after vigorously shaking pentan-1-ol (85 cm^3) and ammonia (specific gravity 0.880, 15 cm^3) together and then allowing them to stand. All solvent systems were prepared fresh daily, and not more than two developments were carried out in any one preparation.

After development, reactant and product spots were visualized under UV light, scraped off the plates, and extracted into a small volume of redistilled methanol, which was evaporated to dryness under reduced pressure. The residual material was identified spectroscopically.

RESULTS AND DISCUSSION

The results relating to the identification of the products formed by I photolysis are summarized in Table I. These products arise principally as a result of bond cleavages in the N–NH–CO–C linkage.

[1] Mass spectra were recorded on an A.E.I. MS9 spectrometer, IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer, NMR spectra were recorded on a Perkin-Elmer R.10 spectrometer, and UV spectra were recorded on a Unicam SP 8000 spectrophotometer.
[2] Les Laboratories, Servicer, Gidy, France.
[3] Servier Laboratories, Greenford, Middlesex, England.
[4] Stanton.
[5] Merck GF_{254}.

0022-3549/79/0800-1063$01.00/0

Table I—Products from Photolytic and Thermal Decomposition of Indapamide

Photochemical Decomposition in N_2-Flushed Methanol	Photochemical Decomposition in O_2-Flushed Methanol	Thermal Decomposition	Product R_f Value[a]	Method of Structure Assignment[b]
II	II	II	0.19[c]	NMR, IR, UV, and mass spectra
III	III	III[d]	0.68	Comparison of IR, UV, and mass spectra with those of a reference sample
		IV	0.75	Comparison of IR, UV, and mass spectra with those of a reference sample
		V[d]	0.26	UV and mass spectra
VI	VI	VI	0.19[c]	NMR, IR, UV, and mass spectra
VII[e]	VII[e]		0.35	Mass spectrum[f]
	VIII[e]		0.45, 0.44*	Mass spectrum[f]
	IX		0.12*	Comparison of IR, UV, and mass spectra with those of a reference sample
	X		0.65	NMR, IR, UV, and mass spectra
	XI[e]		0.27*	Mass spectrum[f]

[a] The R_f values refer to qualitative TLC plates developed in Solvent System 1, except for those marked with an asterisk, which refer to Solvent System 2. [b] Unless otherwise stated, spectra were obtained for products isolated from each of the three decomposition systems. [c] The product at R_f 0.19 was a mixture of II and VI. Compound VI was sublimed out of the mixture and obtained as a white crystalline compound. [d] Product only observed in those thermal decomposition samples maintained at 453°K for 12 hr. [e] Minor product as judged by quantity of material isolated from TLC plates as compared to other products. [f] Only sufficient material could be isolated for a mass spectrum to be recorded.

Type A cleavage leads to 3-sulfamoyl-4-chlorobenzamide (II and III), type B cleavage leads to 3-sulfamoyl-4-chlorobenzoic acid, and type C cleavage yields 1-(*N*-formamido)-2-methylindoline (V) and 1-aminocarboxymethyl-2-methylindoline (VI). Methyl-3-sulfamoyl-4-chlorobenzoate (VIII) presumably arises as a consequence of esterification of VII by the solvent, while IX arises as a consequence of indoline ring oxidative cleavage in species such as III or I. At present, the origin of IV is not clear.

Thermal decomposition of I yields only II–IV besides 2-methylindole (X) and *N*-(3-sulfamoyl-4-chlorobenzamido)-2-methylindole (XI). Thus, type A cleavage can occur from vibrationally excited levels of the ground (S_0) state, and II and III can arise by this mechanism in photolytic reactions. Such species could be generated by internal conversion from the photolytically generated S_1 state. Presumably, the appropriate S_0 levels for dehydrogenation are not accessible *via* internal conversion. Type B and C cleavages only occur under photolysis and must, therefore, arise from the first excited states. Since such reactions are not inhibited by oxygen, which is known to quench triplet-state molecules (4), the S_1 state is implicated.

Thus, the study has demonstrated the complexity of photolysis of a simple pharmaceutical and has shown the wide range of compounds to be screened in photosensitization tests of such molecules.

REFERENCES

(1) D. E. Moore, *J. Pharm. Sci.*, **66**, 1282 (1977).

(2) H. J. Dargie and C. T. Dollery, in "Meyler's Side Effects of Drugs," vol. 8, M. N. G. Dukes, Ed., Excerpta Medica, Amsterdam, The Netherlands, 1975, p. 484.

(3) J. Kynel, K. Oheim, T. Seki, and A. Solles, *Arzneim.-Forsch.*, **25**, 1491 (1975).

(4) S. P. McGlynn, T. Azumi, and M. Kinoshita, "Molecular Spectroscopy of the Triplet State," Prentice-Hall, Englewood Cliffs, N.J., 1969.

ACKNOWLEDGMENTS

The authors thank Mr. D. Sen for experimental assistance.

Improved Spectrophotometric Determination of Glycerol and Its Comparison with an Enzymatic Method

ROY J. STURGEON ˣ, ROBERT L. DEAMER, and HARRY A. HARBISON

Received November 9, 1978, from *McGaw Laboratories, Irvine, CA 92714.* Accepted for publication February 5, 1979.

Abstract □ A chemical method for the determination of glycerol was developed and compared to an enzymatic assay for sensitivity and reproducibility. The chemical assay is based on glycerol oxidation to formaldehyde and subsequent reaction with chromotropic acid to yield a colored product. With this method, as little as 5 μg of glycerol/ml can be detected. The enzymatic assay is based on enzymatic glycerol phosphorylation followed by glycerol phosphate dehydrogenation by nadide (nicotinamide adenine dinucleotide). The reduced nadide is used to reduce iodonitrotetrazolium violet to its colored formazan product. The enzymatic method can be used to determine 50 μg of glycerol/ml in aqueous samples.

Keyphrases □ Glycerol—analysis, spectrophotometry, comparison with enzymatic method □ Spectrophotometry—analysis, glycerol, comparison with enzymatic method

Glycerol is widely used in pharmaceuticals as a vehicle, sweetening agent, emollient, and humectant. Various analysis methods for glycerol have been reported (1–5), but they are usually nonspecific and are limited to a narrow concentration range that gives linear results. Enzymatic methods (2, 4, 5) for glycerol and glycerol-releasing compounds (*i.e.*, triglycerides) have been used widely but are usually expensive and require rigid temperature control and timing of reagent addition to obtain reproducible results.

Chemical methods (1, 3) usually utilize glycerol oxidation to formaldehyde and subsequent reaction with a chromogenic reagent. These methods generally have been shown to produce linear results over a very limited concentration range. The present investigations were undertaken to develop a chemical analysis for glycerol with improved reaction conditions and to compare this method with a standard enzymatic assay.

EXPERIMENTAL

Instrumentation—All absorbance measurements were taken on a

0022-3549/79/0800-1064$01.00/0