(6) V. C. Stephens, C. T. Pugh, N. E. Davis, M. M. Hoehn, R. Ralston, M. C. Sparks, and L. Thompkins, *J. Antibiot.*, **22,** 551 (1969).
(7) C. M. Kunin, *Clin. Pharmacol. Ther.*, **7,** 166(1966).
(8) G. A. J. van Os, E. J. Ariëns, and A. M. Simonis, in "Molecular Pharmacology," E. J. Ariëns, Ed., Academic, New York, N. Y., 1964, p. 30.
(9) C. J. Rammelkamp, *Proc. Soc. Exp. Biol. Med.*, **51,** 95 (1942).
(10) D. C. Grove and W. A. Randall, in "Medical Encyclopedia," New York, N. Y., 1955, p. 96.
(11) G. N. Rolinson and R. Sutherland, *Brit. J. Pharmacol. Chemother.*, **25,** 638(1965).
(12) J. F. Douglas, W. H. Bradshaw, B. J. Ludwig, and D. Powers, *Biochem. Pharmacol.*, **13,** 537(1964).
(13) L. Dettli, *Arzneim.-Forsch.*, **11,** 861(1961).
(14) K. H. Spitzy and G. Hitzenberger, *Antibiot. Annu.*, **1957–1958,** 996.
(15) F. H. Dost and E. Gladtke, *Z. Klin. Chem.*, **1,** 14(1963).
(16) R. G. Wiegand and J. D. Taylor, *Biochem. Pharmacol.*, **3,** 256(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 26, 1971, from the *Experimental Therapy Division and the Pharmaceutical Products Division, Abbott Laboratories, North Chicago, IL 60064*

Accepted for publication December 16, 1971.

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, March 1971.

▲ To whom inquiries should be directed.

DRUG STANDARDS

NMR Stability Assay for Amyl Nitrite Ampuls

R. E. SCHIRMER▲, R. E. ZEMER, and G. G. COOKE

Abstract □ The percentage of amyl nitrite in the contents of an amyl nitrite ampul is determined from the ratio of the area under the $-CH_2-ONO$ line to the area under the $-CH_3$ lines in the NMR spectrum of the sample. The relative standard deviation of the method is 1.4%, and the accuracy is better than 2%.

Keyphrases □ Amyl nitrite ampuls—NMR stability assay □ NMR spectroscopy—stability assay, amyl nitrite ampuls

Amyl nitrite is a highly volatile vasodilator which is used as an inhalant. Many analytical procedures for amyl nitrite have been reported in the literature, including potentiometric determination (1), GC determination (2), colorimetric determinations based on diazotization and coupling of sulfanilic acid with *N*-(1-naphthyl)ethylenediamine (3, 4) and on reaction with ferrous sulfate in sulfuric acid (5), and others (6, 7). NF XIII specified a nitrometric procedure in the past (8) but recently described a GC method (9). No direct spectroscopic method of analysis has been reported for this compound, although its IR (10), UV (11, 12), and 30-MHz. NMR spectra have been studied (13).

The reported methods are not generally well suited for use as stability assays for amyl nitrite ampuls, because most of them are lengthy and special problems are encountered due to the instability and volatility of the compound. In addition, many of the reported methods do not distinguish amyl nitrite from one or more of its many degradation products. The decomposition of amyl nitrite in ampuls has been shown to produce N_2, N_2O, NO, CO, CO_2, and at least 12 liquid components including water, amyl alcohol, isovaleric acid, isovaleraldehyde, amyl isovalerate, and amyl nitrate (14–18). In this paper, an NMR stability assay which is very rapid and which avoids most of the difficulties associated with previous methods is reported.

EXPERIMENTAL

A filled ampul was cleaned on the outside, dried, and weighed. The ampul was then placed in a 10-ml. conical flask with about 0.3 ml. of 0.5% tetramethylsilane in deuterochloroform; it was cracked with a glass rod, and the contents of the flask were swirled briefly. The chloroform solution was placed in a precision NMR tube, the tube was capped, and the 60-MHz. NMR was recorded on an NMR spectrometer[1]. The spectrum was integrated six times, and the ratio of the integral of the $-CH_2ONO$ signal (triplet at 4.70 p.p.m.) to the integral of the methyl proton signals (0.90–1.00 p.p.m.) was computed for each repetition. The percent of amyl nitrite in the ampul is given by:

$$\% \text{ amyl nitrite} = 3 \times \text{mean ratio} \times 100 \qquad \text{(Eq. 1)}$$

The glass from the cracked ampul was washed, dried, and weighed to determine the original fill weight of the ampul.

DISCUSSION

The decomposition of amyl nitrite does not alter the number of methyl groups present, but it does reduce the number of $-CH_2-ONO$ groups (14–18). This allows the degradation of the compound

[1] Varian A-60.

Table I—Chemical Shifts of Protons in Amyl Nitrite Degradation Products (p.p.m. from Tetramethylsilane in $CDCl_3$)

$$(CH_3)_2\overset{4}{C}H-\overset{3}{C}H_2-\overset{2}{C}H_n\sim\overset{1}{X}$$

Compound	Protons 1	Protons 2 and 3	Protons 4
Amyl nitrite	4.70, J_{12} = 6.7 Hz.	1.42–1.84	0.94, J_{34} = 5.5 Hz.
Amyl nitrate	4.50, J_{12} = 6.5 Hz.	1.46–2.00	0.96, J_{34} = 5.6 Hz.
Amyl alcohol	3.60, J_{12} = 6.7 Hz.	1.25–1.98	0.91, J_{34} = 5.4 Hz.
Isovaleraldehyde	9.73, J_{12} = 2 Hz.	1.33–1.58	0.97, J_{34} = 6.0 Hz.
Isovaleric acid	—	1.83–2.50	0.97, J_{34} = 6.0 Hz.
Amyl isovalerate	3.92, J_{12} = 2 Hz.	1.25–2.20	0.88–1.00

to be followed by determining the ratio of the number of $-CH_2ONO$ groups to the number of methyl groups. This NMR procedure does not require any quantitative transfer of the volatile material; it is rapid and specific and as precise as most wet chemical methods. The relative standard deviation of the NMR method was determined to be 1.4% by performing five integrations on each of three different samples. The accuracy of the procedure could not be determined because amyl nitrite could not be obtained in pure enough form to prepare accurate standards, but the accuracy would be expected to be better than 1 or 2% (19).

The NMR data in Table I show that the high field line of the $-CH_2NO_2$ triplet of amyl nitrite overlaps the low field line of the $-CH_2NO_3$ triplet of amyl nitrate in spectra observed at 60 MHz. The overlap would cause the percent amyl nitrite in the sample to be overestimated by 0.25× (percent amyl nitrate in sample). This error is not significant in practice because the amount of amyl nitrate produced is too small to be detected by NMR [the principal degradation products are amyl alcohol, isovaleric acid, and amyl isovalerate (15)]. However, the analyst should be aware of the possibility of interference from amyl nitrate and should examine the region around 4.50 p.p.m. in each spectrum to confirm that the level of the nitrate is in fact negligible. If the spectra are observed at 100 MHz., the $-CH_2NO_2$ and $-CH_2NO_3$ resonances do not overlap and there is no interference from the degradation products.

Table II—Photochemical Degradation of Amyl Nitrite

Exposure Time, min.	Amyl Nitrite Remaining, %
0	98.6
10	96.8
15	91.1
20	90.6

As final evidence that the method will follow the degradation of amyl nitrite, four glass ampuls of the compound were placed 10 cm. in front of a lamp (Hanovia) for varying periods of time, and the contents were then analyzed using this procedure. The results are presented in Table II and show the anticipated degradation. The degradation is relatively slow because the soft glass of the ampul provides some protection for the contents from UV light and because a stabilizing agent was present in the ampuls studied.

REFERENCES

(1) S. Dzottsoti, *Azerb. Khim. Zh.*, **2**, 81(1961); through *Chem. Abstr.*, **57**, 2337h(1962).

(2) N. Iconomou, J. Büchi, and H. Schumacher, *Pharm. Acta Helv.*, **38**, 102(1963).

(3) A. P. Altshuller and C. A. Schwab, *Anal. Chem.*, **31**, 314 (1959).

(4) M. Maruyama and Y. Morotomi, *Tekamine Kenyusho Nempo*, **11**, 122(1959); through *Chem. Abstr.*, **55**, 3928c(1961).

(5) A. F. Fursov, *Aptech. Delo*, **10**, 9(1961); through *Chem. Abstr.*, **55**, 26370b(1961).

(6) G. Takar and I. Simonyi, *Acta Pharm. Hung.*, **27**, 20(1957).

(7) J. Büchi and R. Alther, *Pharm. Acta Helv.*, **31**, 357(1956).

(8) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, pp. 48, 49.

(9) "Second Supplement to the National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1971, pp. 1064, 1065.

(10) L. J. Bellamy and R. L. Williams, *J. Chem. Soc.*, **1957**, 863.

(11) D. H. Szulczewski, M. Yunker, and T. Higuchi, *J. Amer. Pharm. Ass., Sci. Ed.*, **45**, 776(1956).

(12) A. P. Altshuller, I. Cohen, and C. M. Schwab, *J. Phys. Chem.*, **62**, 621(1958).

(13) W. D. Phillips and C. E. Looney, *Mol. Spectrosc.*, **1**, 35 (1957).

(14) D. H. Szulczewski and T. Higuchi, *Anal. Chem.*, **29**, 1541 (1957).

(15) M. H. Yunker, D. Szulczewski, and T. Higuchi, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 613(1958).

(16) *Ibid.*, **47**, 621(1958).

(17) M. H. Yunker, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1957.

(18) D. H. Szulczewski, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1959.

(19) E. D. Becker, "High Resolution NMR," Academic, New York, N. Y., 1969, pp. 236–238.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 7, 1971, from *Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication December 30, 1971.

▲ To whom inquiries should be directed.