(3) Davis, W. M., and Webb, O. L., *Experientia*, **20**, 291(1964).
(4) Truitt, E. B., Jr., Ebersberger, E. M., and Ling, A. S. C., *J. Pharmacol. Exptl. Therap.*, **129**, 445(1960).
(5) Adler, M. W., *ibid.*, **148**, 131(1965).
(6) McQuarrie, D. G., and Fingl, E., *ibid.*, **124**, 264(1958).
(7) Swinyard, E. A., Clark, L. D., Miyahara, J. T., and Wolf, H. H., *ibid.*, **132**, 97(1961).
(8) Lanciault, G., and Wolf, H. H., *J. Pharm. Sci.*, **54**, 841(1965).
(9) Jaffe, J., and Sharpless, S. K., *J. Pharmacol. Exptl. Therap.*, **150**, 140(1965).
(10) Orloff, M. J., Williams, H. L., and Pfeiffer, C. C., *Proc. Soc. Exptl. Biol. Med.*, **70**, 254(1949).
(11) Goldstein, A., "Biostatistics," The Macmillan Co., New York, N. Y., 1964, p. 187.
(12) Tainter, M. L., Tainter, E. G., Lawrence, W. S., Neuru, E. N., Lackey, R. W., Luduena, F. P., Kirtland, H. B., and Gonzalez, R. I., *J. Pharmacol. Exptl. Therap.*, **79**, 42(1943).
(13) Frey, H. H., *Acta Pharmacol. Toxicol.*, **21**, 290(1964).
(14) Longo, V. G., and Silvestrini, B., *J. Pharmacol. Exptl. Therap.*, **120**, 160(1957).
(15) Xavier, E., and Timo-Iaria, C., *Arch. Intern. Pharmacodyn.*, **147**, 512(1964).

Determination of Carbonyl Compounds by Sodium Nitrite Titration of Excess 2,4-Dinitrophenylhydrazine in the Presence of Hydrazone

By LEON KURLANSIK and EDWARD F. SALIM

Numerous methods have been developed for the quantitative determination of carbonyl compounds with 2,4-dinitrophenylhydrazine. Generally, it is necessary to separate the prepared hydrazone from excess reagent before final measurement. A simplified method for the determination of aldehydes and ketones is presented in which excess 2,4-dinitrophenylhydrazine in the presence of hydrazone is titrated with standard sodium nitrite solution.

Since Mathewson (1) prepared hydrazones of water-soluble carbonyl compounds with 2,4-dinitrophenylhydrazine, this reagent has been used extensively for qualitative characterization and quantitative estimation of carbonyl compounds. Applications of gravimetric (2–4), spectrophotometric (5–7), and titrimetric procedures for quantitative determinations of aldehydes and ketones have been reported. Among the titrimetric methods developed are solution of the hydrazone in standard base and determination of excess sodium hydroxide (8), nonaqueous titration of hydrazone in pyridine with tetrabutylammonium hydroxide (9), determination of reduced nitro-groups of the hydrazone (10) or excess hydrazine (11), and direct amperometric titration of the carbonyl with 2,4-dinitrophenylhydrazine solution (12).

A common feature of most published methods is the separation of prepared hydrazone from excess reagent before the final measurement is conducted. The preponderance of procedures deals with the hydrazone and relatively few involve the determination of excess reagent. Isolation of hydrazones which are slightly soluble in the reaction media generally results in low recoveries.

Vulterin and Zyka (13) have described the potentiometric titration of 2,4-dinitrophenylhydrazine with 0.1 *M* sodium nitrite and have postulated the reaction to proceed by formation of the 2,4-dinitrophenylnitrosohydrazine. Since the β nitrogen of the hydrazone is substituted, corresponding nitroso addition presumably does not occur. Baldinus and Rothberg have reported the titration of hydrazones with sodium nitrite but only after vigorous treatment with sulfuric acid and tetrahydrofuran (14). Based on this information a simplified method for the determination of carbonyl compounds has been developed by an initial reaction with 2,4-dinitrophenylhydrazine and subsequent titration of excess reagent in the presence of prepared hydrazone using standard sodium nitrite.

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EXPERIMENTAL

All titrations were conducted potentiometrically using a Beckman Expandomatic pH meter equipped with a calomel and platinum electrode system.

Reagents.—*2,4-Dinitrophenylhydrazine Reagent Solution.*—Add 9 Gm. of finely powdered 2,4-dinitrophenylhydrazine (2,4-DNPH) to a stirred mixture of 100 ml. of 85% phosphoric acid, 100 ml. of ethanol, and 20 ml. of sulfuric acid. Stir for 30 min., add 100 ml. of ethanol, and continue mixing for an additional hour. Cool and filter prior to use. (The reagent is approximately 0.1 *M* and is stable for at least 3 weeks.)

Sodium Nitrite, 0.1 M.—Dissolve 7.5 Gm. of sodium nitrite in sufficient water to make 1000 ml. The solution was standardized against U.S.P. sulfanilamide reference standard, previously dried at 105° for 3 hr., by potentiometric titration using calomel *versus* platinum electrodes.

General Procedure.—Transfer about 1 meq. of test compound to a glass-stoppered, 125-ml. conical flask and dissolve or suspend in 20 ml. of ethanol. Add 25.0 ml. of 2,4-DNPH reagent, insert the stopper, and place the flask in a constant-temperature bath maintained at 50–55° for 1 hr. (*Note:* aldehydes are allowed to react at room temperature for the prescribed 1 hr.) Cool and transfer the contents of the flask to a 400-ml. beaker with the aid of 100 ml. of water. Add 10 ml. of hydrochloric acid, 5 Gm. of potassium bromide, and titrate slowly

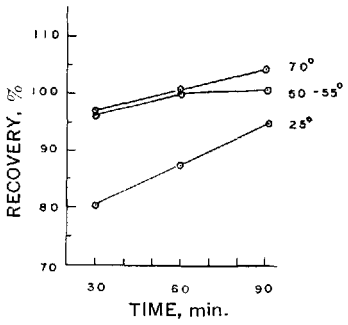


Fig. 1.—Reactivity of methylprednisolone with 2,4-dinitrophenylhydrazine reagent at various temperatures.

with moderate stirring, adding 0.1 *M* sodium nitrite in 0.1-ml. increments near the end point. Perform a blank determination. The difference between blank and sample titrations represents the volume of 0.1 *M* sodium nitrite equivalent to the carbonyl content in the sample.

The end point was determined by means of differential curves ($\Delta E/\Delta$ ml. *versus* ml.). It was noted that the volume of titrant added at the point of maximum rise in potential was the equivalent volume, thereby eliminating the necessity to construct a differential curve for each titration.

RESULTS AND DISCUSSION

Initial studies were conducted using standard 2,4-DNPH reagents. Johnson's reagent (15) consisting of ethanolic phosphoric acid was considered too viscous to allow the uniform additions necessary in the proposed method and was noted to yield quantitative hydrazone formation only after prolonged reaction. The reagent developed by Brady (16) utilizing ethanolic sulfuric acid was observed to accelerate formation of hydrazone but produced undesirable side reactions as demonstrated by high recoveries with methylprednisolone. Experiments using various combinations of phosphoric and sulfuric acids to resolve the difficulties associated with each reagent led to the formulated solution used throughout subsequent investigations.

TABLE I.—DETERMINATION OF ALDEHYDES AS 2,4-DINITROPHENYLHYDRAZONES

	Recovery, %
Benzaldehyde[b]	98.8, 98.4
Cinnamaldehyde[b]	99.4, 99.7
p-Dimethylaminobenzaldehyde[a]	99.4, 99.6
Ethyl vanillin[a]	100.4, 100.6
Formaldehyde solution U.S.P.	100.1[c]
Heptaldehyde	98.5
α-Naphthaldehyde[b]	99.8[d]
p-Nitrobenzaldehyde[a]	98.7
2-Pyridinecarboxaldehyde	99.1
Salicylaldehyde[b]	100.4, 99.5
Vanillin[a]	99.6

[a] Recrystallized before use. [b] Redistilled before use. [c] Calculated on basis of formaldehyde content found by U.S.P. assay. [d] Average of 5 determinations of S.D. ± 0.30.

A supplemental study was performed to determine the optimum time and temperature conditions for reactivity. The results shown in Fig. 1 indicate that the reaction for methylprednisolone was quantitative at 50–55° after 1 hr. The conditions established for methylprednisolone were believed to express the parameters by which sterically unhindered ketones could be readily quantitated. Analogous tests for aldehydes showed that quantitative results could be obtained at room temperature within a 1-hr. period. A summary of these data is included in Table I. For the limited number of aldehydes tested, no pronounced effects were observed for increased number of carbons in aliphatic compounds nor the addition of electron donating or withdrawing groups *ortho* or *para* to the aromatic aldehydes.

The determination of ketones was found to be influenced by certain factors so that the method was not so generally applicable as with the aldehydes. Ketones successfully determined are included in Table II. The general procedure was found to be

TABLE II.—DETERMINATION OF KETONES AS 2,4-DINITROPHENYLHYDRAZONES

	Recovery, %
Benzophenone[a]	99.8
ω-Bromoacetophenone[a]	98.8
Chalcone[a]	100.8
Cyclohexanone[b]	99.0
Dehydrocholic acid[a]	100.1[c]
3,4-Dihydro-1(2H)-naphthalenone	95.8
1,3-Diphenyl-2-propanone[a]	99.8
Estrone	100.4
Menadione[a]	99.1[d]
Methyl isobutyl ketone[b]	96.6, 96.6
Methylprednisolone	99.1, 99.7
Methyltestosterone	99.5
Prednisolone	99.1
Prednisone	100.0[c,e]
Progesterone	100.6

[a] Recrystallized before use. [b] Redistilled before use. [c] Reacted for 3 hr. [d] Calculated on basis of monohydrazone. [e] Recovery based on hydrazone formation at 3 and 20 positions only.

quantitative for mono-ketones above C_5. Lower aliphatic ketones produced the following results: acetone (84.8%), methyl ethyl ketone (89.8%), and methyl isopropyl ketone (93.7%). The low values may be explained on the basis of nitrous acid oxidation of methyl or methylene groups adjacent to a carbonyl group. In aqueous HCl solution the reaction proceeds to the formation of a 1,2-dicarbonyl compound and hydroxylamine hydrochloride (17). The hydrazones of lower molecular weight ketones contain the grouping CH_3—C=N— which is the nitrogen analog of the carbonyl group and may undergo a similar reaction during the final titration with sodium nitrite.

Several steroids were included in this general survey because of the varied positions occupied by carbonyls and in light of the wide use of such compounds as medicinal agents. Steroids containing a keto group in the 3 or 17 position were quantitative in the alloted reaction time. Dehydrocholic acid

with carbonyls in the 3, 7, and 12 positions required 3 hr. at 50–55° for quantitative reactivity. Prednisone, a 3,11,20-trione compound, gave 66.7% recovery after 3 hr. and no further reaction was evident after 17 hr. elapsed time. Since the 11 keto position is known to be extremely unreactive, the result at 3 hr. can be considered quantitative based on hydrazone formation at the 3 and 20 positions only. Progesterone, prednisolone, and methylprednisolone which represent 3,20 di-ketones yielded quantitative bis-hydrazones. Prednisolone, esterified as the acetate, gave an incomplete reaction—81.5%—after 3 hr. The presence of the bulkier substituent adjacent to carbon 20 appears to be sufficient to drastically hinder the formation of hydrazone at the C_{20} carbonyl.

SUMMARY

The titration of excess 2,4-dinitrophenylhydrazine using sodium nitrite without prior separation of the precipitated hydrazone allows a shorter analysis time for the determination of aldehydes and a substantial number of ketones. The procedure was found to be reproducible to ±0.5% and the results for ethyl vanillin, formaldehyde solution U.S.P., prednisolone, menadione, and prednisone were consistently within 0.5% of comparative values obtained by official or other recognized methods of analysis.

The method is neither applicable to certain aliphatic ketones nor to sterically hindered steroids. Carbonyl compounds which contain a functional group such as an amine or phenol capable of reacting with sodium nitrite cannot be determined by the proposed procedure.

REFERENCES

(1) Mathewson, W. E., *J. Am. Chem. Soc.*, **42**, 1277 (1920).
(2) Fernandez, O., and Socias, L., *Rev. Acad. Cienc. Madrid*, **28**, 330(1932); through *Chem. Abstr.*, **26**, 4556 (1932).
(3) Iddles, H. A., and Jackson, C. E., *Ind. Eng. Chem., Anal. Ed.*, **6**, 454(1934).
(4) Iddles, H. A., Low, A. W., Rosen, B. D., and Hart, R. T., *ibid.*, **11**, 102(1939).
(5) Lappin, G. R., and Clark, L. C., *Anal. Chem.*, **23**, 541(1951).
(6) Lohman, F. H., *ibid.*, **30**, 972(1958).
(7) Monty, K. J., *ibid.*, **30**, 1350(1958).
(8) Clift, F. P., and Cook, R. P., *Biochem. J.*, **26**, 1800 (1932).
(9) Sensabaugh, A. J., Cundiff, R. H., and Markunas, P. C., *Anal. Chem.*, **30**, 1445(1958).
(10) Espil, L., and Mandillon, G., *Compt. Rend. Soc. Biol.*, **129**, 1187(1938).
(11) Schoniger, W., and Lieb, H., *Mikrochemie ver. Microchim. Acta*, **38**, 165(1951).
(12) Zobov, E. V., and Lyalikov, Yu. S., *Zh. Analit. Khim.*, **11**, 459(1956); through *Chem. Abstr*, **51**, 14488 (1957).
(13) Vulterin, J., and Zyka, J., *Chem. Listy*, **50**, 364(1956).
(14) Baldinus, J. G., and Rothberg, I., *Anal. Chem.*, **34**, 924(1962).
(15) Johnson, G. D., *J. Am. Chem. Soc.*, **73**, 5888(1951).
(16) Brady, O. L., *J. Chem. Soc.*, **1931**, 756.
(17) Royals, E. E., "Advanced Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1954, p. 568.

Amides Derived from Hepta- and Octamethyleneimine

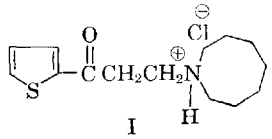
By HEINO A. LUTS, W. A. ZUCARELLO*, W. LEWIS NOBLES†, and JEROME F. GRATTAN

Some new physiologically active amides of hepta- and octamethyleneimine moiety have been prepared. Their preparation and biological activities are given.

THE KNOWLEDGE that a number of biologically important compounds occurring in nature contain trimethoxyphenyl or trimethoxybenzoyl groups as a part of their molecule has prompted considerable investigation into the various ways which this moiety can be incorporated into molecules and elicit various pharmacological actions.

Vargha and his associates (1) have reported on the tranquilizing and analgesic effects of the simple benzamide containing the above moiety as well as a number of heterocyclic amides. In varying the amine moiety of the amide, they noted that morpholine and 2-methylmorpholine exhibited the most desirable therapeutic properties among the compounds studied. Correspondingly, Schlager (2) in a review article has reported on a large number of derivatives containing 3,4,5-trimethoxybenzoyl moiety.

Previously, the authors (3) have reported on the use of heptamethyleneimine in the Mannich reaction. It may be worthy of note that in this earlier work, the Mannich base obtained from 2-acetylthiophene and heptamethyleneimine exhibited significant analgesic activity at a dosage level of 150 mg./Kg.



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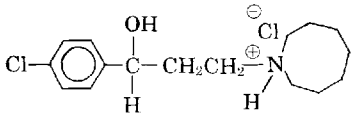

II

In addition, the secondary alcohol obtained by the sodium borohydride reduction of the Mannich base from *p*-chloroacetophenone and heptamethyleneimine

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