acids, though the balance between the essential amino acids is much better than in wheat. Similarly, opaque-2 is superior to normal corn. In agreement with feeding tests of Sure (1955), the chemical amino acid assays indicate that buckwheat has a better balance and better potential than cereal grains of supplementing foods which are low in lysine.

There is presumptive evidence that concentrations of the essential amino acids of egg protein are higher than concentrations required by man (FAO/WHO/UNICEF, 1970). Isoleucine and methionine are particularly high, and the use of egg as a reference may overestimate the extent to which those amino acids are limiting and may underestimate the quality of a protein for human use. Consequently, the nutritional value of opaque-2 corn, oat, and buckwheat proteins is probably higher than indicated in the data given in Table VI. On the other hand, amino acid analyses do not measure one of the most important parameters that determine nutritive value of a food, its digestibility, and "chemical scores" should be considered primarily as a powerful and convenient screening tool.

ACKNOWLEDGMENT

The technical assistance of J. T. Gilbertson with the amino acid analyses is gratefully acknowledged. We thank J. W. Bailey (The Birkett Mills, Pen Yan, N.Y.) for the commercially milled samples, and H. G. Marshall for the samples of buckwheat and for useful suggestions in preparation of the manuscript.

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Received for review June 11, 1971. Accepted September 8, 1971. This work was a cooperative investigation of the Plant Science Research Division, Agricultural Research Service, USDA, and the Wisconsin Agricultural Experiment Station, Madison, Wisconsin. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Spectral and Gas Chromatographic Characteristics of Some *N*-Nitrosamines

John W. Pensabene,* Walter Fiddler, Calvin J. Dooley, Robert C. Doerr, and Aaron E. Wasserman

The mass spectral, infrared spectral, and gas–liquid chromatographic retention time data of 25 *N*-nitrosamines are reported, together with general procedures for the synthesis of *N*-nitrosamines not commercially available.

Since the discovery of the toxic and later the carcinogenic properties of dimethylnitrosamine (Barnes and Magee, 1954; Magee and Barnes, 1956) many investigators have been working with nitrosamines. The most extensive review on the properties of *N*-nitroso compounds has been reported by Druckrey *et al.* (1967).

The report of dimethylnitrosamine and its causal relationship to liver damage in sheep (Ender *et al.*, 1964) has raised the question of the occurrence of various nitrosamines in the food supply. The only confirmed reports of a nitrosamine in food products have been those of dimethylnitrosamine in herring fish meal (Ender *et al.*, 1964), in a South African Bantu food plant (DuPlessis *et al.*, 1969), and in various fish products (Fazio *et al.*, 1971). However, there have been other reports of the presence of nitrosamines in foods: wheat meal (Kroeller, 1967; Marquardt and Hedler, 1966), cheese (Freimuth and Gläser, 1970; Kroeller, 1967; Marquardt and Hedler, 1966), milk (Marquardt and Hedler, 1966), mushrooms (Ender and Ceh, 1968), African alcoholic beverage (McGlashan *et al.*, 1968), meat products (Ender and Ceh, 1968; Freimuth and Gläser, 1970), and fish products (Ender and Ceh, 1968; Howard *et al.*, 1970; Sen *et al.*, 1970). The accuracy of many of these reports is questionable, as specific methods for con-

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firming the identity of the nitrosamines were not utilized and a number of other food components are known to appear as artifacts in the analyses.

Analytical procedures for nitrosamines in foods must include unambiguous confirmation of identity. The best unequivocal technique known at present is mass spectrometry, which is a major technique in structural elucidation because of its sensitivity and capability in analysis of compounds in mixtures.

Although *N*-nitrosamines are not uncommon compounds, very little correlated mass spectral, infrared, and glc information is available in the literature. Collin (1954) reported the mass spectra of four symmetrical dialkylnitrosamines ranging from dimethyl through dibutyl. Schroll *et al.* (1967) investigated these same compounds together with several substituted *N*-nitrosoanilines and alicyclic nitrosamines. Ultraviolet data and some other physical data were listed for many *N*-nitrosamines by Druckrey *et al.* (1957). However, data are not available for many nitrosamines which are not commercially obtainable.

Therefore, in view of our interest and that of others in the study of these compounds in foods, this paper is a convenient compilation for reference of some of the physical properties of *N*-nitrosamines such as boiling points, gas–liquid chromatographic, infrared, and mass spectral data, most of which are not available in the literature.

EXPERIMENTAL

Synthesis of Nitrosamines. Precautions should be taken in the handling of these compounds to prevent inhalation and exposure to the skin, since they have potentially toxic properties.

N-Nitrosamines were prepared from the appropriate secondary amine and either nitrosyl tetrafluoroborate or sodium nitrite.

NITROSYL TETRAFLUOROBORATE. *N*-Nitroso-*N*-methylbutylamine, *N*-nitroso-*N*-methylbenzylamine, *N*-nitroso-*N*-ethylbenzylamine, and *N*-nitroso-*N*-butylaniline were prepared according to the procedure described by Olah *et al.* (1956).

To an absolute ether solution of 1 mol of the secondary amine is added 0.5 mol of nitrosyl tetrafluoroborate in small portions with vigorous stirring and cooling with ice. After addition is completed, the mixture is stirred for an additional 10 min. Two layers appear. The ethereal yellow upper layer contains the nitrosamine. The layers are separated and the ether solution is dried over anhydrous sodium sulfate, filtered, concentrated under a stream of nitrogen, and then fractionally distilled *in vacuo*.

SODIUM NITRITE. *N*-Nitrosodiallylamine, *N*-nitroso-*N*-methylethylamine, *N*-nitroso-*N*-methylpropylamine, *N*-nitroso-*N*-ethylpropylamine, *N*-nitroso-*N*-ethylbutylamine, *N*-nitroso-*N*-propylbutylamine, *N*-nitrosodiamylamine, *N*-nitroso-*N*-ethylaniline, *N*-nitroso-*N*-propylaniline, and *N*-nitrosomorpholine were prepared as follows. An equimolar secondary amine–hydrochloric acid solution is cooled with ice. To this, a twofold excess of an aqueous solution of sodium nitrite is added. After addition has been completed, the reaction mixture is heated at 60°C for 1 hr and then cooled. The nitrosamine, separating as a yellow oil, is extracted three times with ether and the combined extracts are dried over anhydrous sodium sulfate, filtered, concentrated under a stream of nitrogen, and fractionally distilled *in vacuo*.

N-Nitroso-*N*-methylethanolamine was prepared as reported by Ogimachi and Kruse (1961).

The other *N*-nitrosamines used in this study were obtained from commercial sources.

All samples were checked for purity by gas chromatography prior to analysis and, when necessary, pure material was collected by preparative gas chromatographic procedures.

Gas–Liquid Chromatography. A Perkin-Elmer model 800 dual column gas chromatograph equipped with flame ionization detectors was used. The detector and injector port temperatures were 250 and 195°C, respectively. A 10-ft × $^1/_8$-in. o.d. stainless steel column containing 5% Carbowax 20M-TPA on 100–120 mesh Supelcoport was used with a helium carrier flow of 60 cm^3 per min. The air and hydrogen flows were 430 and 24 cm^3 per min, respectively. Temperature program: 5 min isothermal at 100°C, then 100 to 185°C at 5°C per min. A 10-ft × $^1/_8$-in. o.d. stainless steel column containing 5% OV-1 on 35 to 50 mesh Anakrom ABS was used with a helium carrier flow of 118 cm^3 per min. The air and hydrogen flows were 430 and 40 cm^3 per min, respectively. Temperature program: 70 to 185°C at 5°C per min. The retention times for even C_6–C_{16} methyl esters of straight chain acids are given to indicate where the nitrosamines would elute under other conditions.

Infrared Spectra. The nitrosamines were dissolved in carbon tetrachloride and the spectra recorded in a 0.5-mm pathlength NaCl cell with a Perkin-Elmer Model 421 infrared spectrophotometer. Due to its lack of solubility in a suitable solvent and poor spectra as a film or pellet, no ir is presented for *N*-nitroso-*N*-methylethanolamine.

Mass Spectra. Mass spectra were obtained with a Du Pont model 21-492 mass spectrometer at an ionizing voltage of 70 eV and an ion source temperature of 210°C. The volatile liquids were introduced *via* the bath inlet heated at 150°C. The relatively nonvolatile liquids and solids were introduced *via* the solid probe at the lowest temperature required to produce volatility.

RESULTS AND DISCUSSION

N-Nitrosamines were prepared from secondary amines by two methods—reaction with sodium nitrite or nitrosyl tetrafluoroborate. The latter method is preferred because, with ether-soluble amines, this simple reaction proceeds without the formation of secondary reaction products.

The gas chromatographic retention times for the *N*-nitrosamines are listed in Table I, together with their boiling or melting points. The simple aliphatic compounds were readily separated on a Carbowax 20M column, but difficulty was experienced with aromatic nitrosamines under the same conditions.

There was significant decomposition of the phenyl-substituted compounds, methyl through butyl, on the Carbowax 20M column with the formation of the corresponding aniline and asymmetrical hydrazines. This decomposition occurred to a lesser extent on the OV-1 column, which, with modification of the temperature program, permitted satisfactory separations. A retention value could not be obtained for *N*-nitrosophenylbenzylamine since there were indications that it is more labile than the other alkyl- and benzyl-substituted nitrosamines, possibly because nitric oxide is split off thermally as reported by Welzel (1971) for diaryl nitrosamines.

The gas chromatographic data were obtained with $^1/_8$-in. packed columns and the resolution was compared favorably with the separations reported by Heyns and Roper (1970) of other nitrosamines using an alkaline UCON LB 550X capillary column.

Where sufficient material is available for collection and

Table I. Gas Chromatographic Data and Boiling or Melting Points

RR'N—N=O

R	R'	Molecular formula	Mol wt	bp or mp	CW20M-TPA RT, min	OV-1 RT, min
Me	Me	$C_2H_6N_2O$	74	151°C/760 mm	3.0	
Me	Et	$C_3H_8N_2O$	88	161°C/760 mm	3.7	
Me	Pr	$C_4H_{10}N_2O$	102	90°C/40 mm	5.4	
Me	Bu	$C_5H_{12}N_2O$	116	198°C/760 mm	7.8	
$HOCH_2CH_2$	Me	$C_3H_8N_2O_2$	104	110°C/1 mm	22.0	
Et	Et	$C_4H_{10}N_2O$	102	64°C/17 mm	4.3	
Et	Pr	$C_5H_{12}N_2O$	116	104°C/44 mm	6.0	
Et	Bu	$C_6H_{14}N_2O$	130	95°C/14 mm	8.4	
Pr	Pr	$C_6H_{14}N_2O$	130	81°C/5 mm	7.8	
Pr	Bu	$C_7H_{16}N_2O$	144	103°C/13 mm	10.0	
Bu	Bu	$C_8H_{18}N_2O$	158	116°C/14 mm	12.3	
Am	Am	$C_{10}H_{20}N_2O$	186	124°C/5 mm	16.4	
Allyl	Allyl	$C_6H_{10}N_2O$	126	41°C/1 mm	8.0	
$-(CH_2)_5-$		$C_5H_{10}N_2O$	114	100°C/14 mm	12.6	
$-(CH_2)_4-$		$C_4H_8N_2O$	100	98°C/12 mm	13.3	
$-(CH_2)_2O(CH_2)_2-$		$C_4H_8N_2O_2$	116	96°C/6 mm	14.5	
$-(CH_2)N(CH_3)(CH_2)_2-$		$C_5H_{11}N_3O$	129	83°C/4 mm	14.8	
Ph	Me	$C_7H_8N_2O$	136	128°C/19 mm	18.2	6.4
Ph	Et	$C_8H_{10}N_2O$	150	131°C/20 mm	18.3	7.6
Ph	Pr	$C_9H_{12}N_2O$	164	109°C/3.5 mm	19.6	9.1
Ph	Bu	$C_{10}H_{14}N_2O$	178	139°C/8.5 mm	21.3	11.5
Ph	$PhCH_2$	$C_{13}H_{12}N_2O$	212	mp 57–58°C	...	...
$PhCH_2$	Me	$C_8H_{10}N_2O$	150	158°C/26 mm	21.2	8.2
$PhCH_2$	Et	$C_9H_{12}N_2O$	164	162°C/23 mm	21.6	9.6
$PhCH_2$	$PhCH_2$	$C_{14}H_{14}N_2O$	226	mp 58–59°C	...	20.0
			Markers			
C_6	Methyl hexanoate				1.7	
C_8	Methyl octanoate				4.0	4.2
C_{10}	Methyl decanoate				9.2	8.5
C_{12}	Methyl dodecanoate				14.1	12.8
C_{14}	Methyl tetradecanoate				18.5	17.1
C_{16}	Methyl hexadecanoate				22.5	21.0

characterization, the infrared spectra may be helpful. Figure 1 shows the infrared spectrum of *N*-nitrosodimethylamine in carbon tetrachloride. Spectra of other *N*-nitrosamines have been deposited with the ACS Microfilm Depository Service. The most distinctive band is the strong $\gamma_{N=O}$ stretch band from 1435 to 1485 cm^{-1}. The alicyclic nitrosamines absorb in the 1440 cm^{-1} region, simple disubstituted aliphatic and benzyl–allyl nitrosamines absorb in the 1460 cm^{-1} region, and phenyl–alkyl nitrosamines absorb in the 1480 cm^{-1} region. This is in substantial agreement with the data reported by Williams *et al.* (1964).

For *N*-nitrosamines present in low concentrations, in complex mixtures, gas–liquid chromatography is the most potent tool for separation and isolation and mass spectrometry for unequivocal confirmation of identity. The mass spectrum of *N*-nitrosodimethylamine is listed in Figure 2 in the form of a bar graph. Spectra of other *N*-nitrosodimethylamines have been deposited with the ACS Microfilm Depository Service.

In general, the fragmentation process of the aliphatic nitrosamines follows the scheme described by Budzikiewicz *et al.*

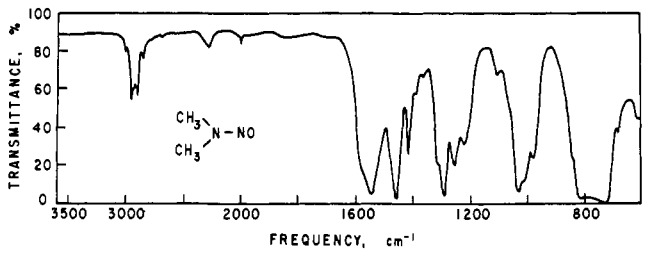


Figure 1. Infrared spectrum of *N*-nitrosodimethylamine in carbon tetrachloride

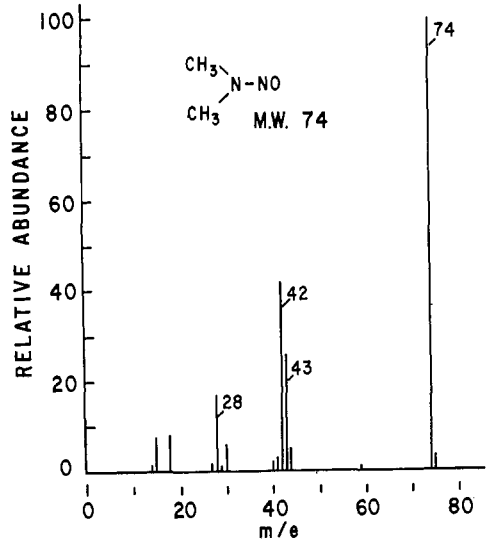
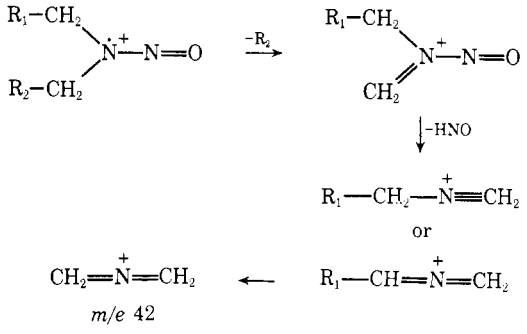


Figure 2. Mass spectrum of *N*-nitrosodimethylamine

(1967) with the important ions being *m/e* 30, 42, a relatively large parent ion and peak due to α-cleavage of the alkyl side chain, with subsequent loss of NOH, as well as a peak at P-17 due to the loss of the hydroxyl radical. An important pathway can be described in the following scheme for the *m/e* 42, except in the case of dimethylnitrosamine, which bypasses the α-cleavage step.



The phenyl-substituted nitrosamines all have an appreciable parent peak and a strong P-30 peak. The benzyl-substituted nitrosamines have a strong parent peak and a strong *m/e* 91 peak, which in some cases is the base peak of the spectrum. The *m/e* 91 is analogous to the splitting of the bond β to the phenyl ring, as described by Grubb and Meyerson (1963).

Currently, a more specific and detailed study is in progress utilizing both high-resolution mass spectrum and the metastable spectrum of each compound. This will allow us to more fully explain the fragmentations that occur.

The determination of *N*-nitrosamines in food products and other natural substances is complicated by the presence of large numbers of interfering compounds. Therefore, adequate isolation and cleanup procedures are necessary to permit sampling of the nitrosamines so that the spectra described in this paper can be utilized.

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Received for review August 24, 1971. Accepted October 28, 1971. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned. Spectra of N-nitrosamines will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D.C. 20036, by referring to author, title of article, volume, and page number. Remit check or money order for $6.00 for photocopy or $2.00 for microfiche.

Processing of Cauliflower Leaf Waste for Poultry and Animal Feed

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Leaf waste from commercially grown cauliflower was dehydrated in a pilot scale alfalfa dehydrator to give dried meals suitable for poultry and cattle feeds. Separation of the poultry and cattle meal fractions was accomplished *via* air classification of the dried plant material. The poultry meal fraction contained 375 to 620 mg/kg of xanthophyll and 26 to 31% protein, while the cattle meal contained 17 to 21% protein. The xanthophylls in the poultry meal were as effective in pigmenting broiler skin as the xanthophylls in dehydrated alfalfa meal. No undesirable flavor was imparted to poultry meat by cauliflower meal. Pressing of cauliflower leaf prior to dehydration increased its solids content and lessened the quantity of water to be evaporated per pound of dried product by nearly 30%. The pressed and dehydrated meals were almost equal in quality to the unpressed dehydrated meals.

Commercial production of vegetables in the United States results in more than 4 million tons of fresh vegetable wastes annually (Willaman and Eskew, 1948; U.S. Dept. Agr. Stat., 1969). A large portion of these wastes is green leaf plant materials that are removed at the packing shed. An estimated 60,000 tons of cauliflower leaf wastes are produced annually in the California Salinas Valley. Presently, following removal of the flowers for the fresh and frozen vegetable markets, leaf and stem portions are chopped and returned to the fields where they constitute an odor problem as they decompose to form a green manure.

Prior studies at this laboratory have been concerned with

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