Table II. Mass Spectrometer Analyses of Synthetic Blend Using 0.001-Ml. Self-Filling Micropipet

(Air-flow method used for calibrations and samples)

Component	Synthetic Composition	Analysis 1	Analysis 2
		Volume %	
n-Nonane	3.2	3.5	3.9
n-Octane	74.8	73.2	75.3
2-Methylheptane	7.0	6.9	7.0
3-Methylheptane	6.9	7.2	6.4
4-Methylheptane	2.9	4.3	2.9
n-Heptane	5.2	4.9	4.5

Calculating time is appreciably decreased by the use of this pipet. It is not necessary to calculate percentage patterns from the spectra of pure calibration compounds because the amount of sample, and, therefore, the spectral peak height, are always the same, within experimental error. For the same reason, sensitivity coefficients (peak heights ÷ sample pressure or volume) are not needed, for the peaks themselves serve this purpose. Because the partial volumes of the constituents add up to near 1.0, approximate percentages are obtained immediately, although normalization to 1.0, or 100%, is usually necessary.

Application with increased accuracy to C_4 alcohols, C_3 acids, and other oxygenated compounds has been obtained by a modification of the mercury seal method, which consists of forcibly squirting sample and mercury through the pipet and onto the sintered disk.

ACKNOWLEDGMENT

The authors wish to thank Sidney Katz, Institute of Gas Technology, Chicago, Ill., for describing the use of the self-filling pipet in microchemistry.

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2-Nitro-1,3-indandione

Promising Reagent for Identification of Organic Bases

B. E. CHRISTENSEN, C. H. WANG, I. W. DAVIES, AND DARRELL HARRIS
Oregon State College, Corvallis, Ore.

IN 1936 Wanag (*4*) described a new acidic reagent, 2-nitro-1,3-indandione, which formed salts with both inorganic cations and organic bases such as the aliphatic and aromatic amines and the nitrogen heterocycles. These derivatives were reported (*4–6*) to be crystalline, nonhygroscopic, water-soluble compounds which, with few exceptions, gave sharp melting points. Rosenthaler (*3*) applied the reagent to the study of alkaloids but other than reporting crystal formation gave no analytical or melting point data. Later Müller (*1*) extended the work to a number of miscellaneous compounds such as histamine, tyramine, and arginine.

2-Nitro-1,3-indandione is a strong acid; hence many of its salts hydrolyze in an aqueous medium to yield acidic solutions. The extent of the hydrolysis depends on the ionization constant of the base used in the preparation, which in most cases is so small as to permit the titration of the free acid, and thus make it possible to determine the neutral equivalent of the salt. This property, unrecognized by Wanag, and not possessed by the usual amine derivatives, gives unusual promise to 2-nitro-1,3-indandione as a reagent for the identification of organic bases. For these reasons the work of Wanag was continued in this laboratory, with particular attention to the acid properties of the salts.

Because only a limited number of heterocyclic derivatives were described by Wanag and others, most of the additional preparative work was devoted to the heterocyclic compounds. The salts were originally prepared by the addition of an aqueous solution of the acid to a dilute hydrochloric acid solution of the amine. As 2-nitro-1,3-indandione is very insoluble in dilute hydrochloric acid, impure salts were often obtained, which required several recrystallizations. Experiments with other solvents indicated that acetone was a more suitable reaction medium, inasmuch as both the organic bases and the free acid were usually soluble in this medium, whereas the salts either precipitated immediately or slowly crystallized. In a few instances the salts precipitated as oils which failed to crystallize.

Except for compounds in which the nitrogen was not functionally basic, such as uracil, xanthine, or acetanilide, all others gave derivatives with this reagent. The alkylamines form crystalline salts which do not hydrolyze sufficiently to give neutralization equivalent measurements. The bulk of the nitrogenous bases, however, gave derivatives which with few exceptions (due to color interference) could be titrated.

In certain cases, such as the titration of the 4-quinazolone derivative, abnormal amounts of alkali were required, owing to the partial neutralization of the enol form of the quinazolone. In almost every instance in which the ionization constants of the bases were known to be less than 10^{-6} the indandione derivative gave a titration value within 2% of theory (with the exceptions cited above).

Although melting point data pertaining to the indandionates are to be found in the literature, earlier workers failed to point out that these values were for the most part decomposition points which are difficult to reproduce, and hence variations of several degrees often were noted among individual observers. However, the derivatives of 2-nitro-1,3-indandione are very easily prepared, and this makes this reagent extremely useful for isolation as well as characterization purposes.

2-Nitro-1,3-indandione was first prepared according to the directions of Wanag (*4, 5*) by the nitration of 1,3-indandione with cold fuming nitric acid in glacial acetic acid under conditions which gave rather erratic yields. In this laboratory it was discovered that more reproducible results were obtained with ordinary concentrated nitric acid containing oxides of nitrogen. These oxides have a marked influence on the course of the reactions, favoring the formation of nitration rather than oxidation products.

EXPERIMENTAL

2-Nitro-1,3-indandione. Dissolve 20 grams of 1,3-indandione in 200 ml. of glacial acetic acid and raise the temperature of solution to 48° C. Add 20 ml. of 50 to 60% nitric acid visibly colored with the oxides of nitrogen. Shake or stir 5 to 10 seconds and place the flask immediately under cold running tap water while continuing the agitation for several additional minutes. After cooling for 30 to 60 minutes, remove as much of the mother liquid as possible by filtration. Dissolve the crude 2-nitro-1,3-indandione in 500 ml. of cold water, decolorize with charcoal at 10° to

Table I. Titration Data

Salts of 2-Nitro-1,3-indandione	M.P. (Dec.) of Salt, ° C.	Neutral Equivalent Theory	Neutral Equivalent Found	Solvent Used	% Carbon Theory	% Carbon Found	% Hydrogen Theory	% Hydrogen Found	% Nitrogen Theory	% Nitrogen Found
Acetamidine	254–7[a]		U.T.T.[b]	Water	...	...	...	...	...	...
Acridine	183	370	373	Acetone	...	...	...	...	...	...
Adenine	...		U.T.T.		51.5	51.3	3.65	3.45	...	...
dl-Alanine	197–8[c]	140	266	Acetone	51.4	51.4	4.32	4.38	...	...
p-Aminoacetanilide	214	341	340	Acetone	...	...	...	...	...	...
p-Aminoacetophenone	191–4	326	332	Acetone	...	...	...	...	...	...
o-Aminoacetophenone	164–6	326	320	Acetone	62.6	62.1	4.32	4.20	...	...
p-Aminoazobenzene	...		U.T.T.	Acetone	...	...	...	...	...	...
m-Aminobenzoic acid	204–5	164	163	Acetone	...	...	...	...	...	...
p-Aminobenzoic acid	203–5	164	164	Acetone	...	...	...	...	...	...
p-Aminobiphenyl	196	360	369	Acetone	...	...	...	...	...	...
o-Aminophenol	...	300	296	Water	...	...	...	...	...	...
p-Aminophenol	...	300	298	Acetone	...	...	...	...	...	...
m-Aminophenol	201–3	300	306	Alcohol	...	...	...	...	...	...
α-Aminopyridine	189	285	284	Acetone	...	...	...	...	...	...
5-Aminouracil.H_2O	...	336	315	Water	46.4	46.8	3.60	3.45	16.7	16.5
Ammonia	222–4	...	U.T.T.	Water	...	...	...	...	...	...
Aniline	185–7	284	284	Acetone	...	...	...	...	...	...
o-Anisidine	176–7	314	312	Acetone	...	...	...	...	...	...
p-Anisidine	191–2	314	313	Acetone	...	...	...	...	...	...
Anthranilic acid.$^3/_2H_2O$	174–5	177	175	Acetone	54.1	54.2	4.26	4.15	7.88	8.11
Benzamidine	189–90	...	U.T.T.	Water	...	...	...	...	...	...
Benzylamine	178–9	...	U.T.T.	Acetone	...	...	...	...	...	...
Bornylamine	224–5	344	347	Alcohol	...	...	...	...	...	...
n-Butylamine	143–4	...	U.T.T.	Acetone	...	...	...	...	...	...
5-Bromo-8-nitroquinoline	138	444	453	Acetone	48.7	48.9	2.27	2.38	...	...
Caffeine	177	385	379	Acetone	53.0	53.2	3.92	3.93	...	...
m-Chloroaniline	179–80	318	317	Acetone	...	...	...	...	...	...
o-Chloroaniline	168–9	318	315	Acetone	...	...	...	...	...	...
p-Chloroaniline	187–9	318	314	Acetone	...	...	...	...	...	...
7-Chloro-4-quinazolone	192	372	314	Acetone	54.9	54.9	2.71	2.73	11.31	11.27
Cinchonine.1H_2O (di salt)	167–8	347	329	Water	64.0	64.2	4.91	5.12	8.06	7.98
Creatine	...	322	329	Water	48.5	48.4	4.38	4.72	...	...
Creatinine	...	304	305	Water	51.3	50.9	3.98	4.06	...	...
Cyclohexylamine	221–2	...	U.T.T.	Acetone	...	...	...	...	...	...
o-Dianisidine	216–17	313	308	Acetone	...	...	...	...	...	...
Diazoaminobenzene (di salt)	176–8	290	287	Acetone	62.2	62.1	3.65	4.31	...	...
Dibenzylamine	202–4	388	381	Acetone	...	...	...	...	...	...
4,7-Dichloroquinoline	156–7	389	386	Acetone	55.6	55.1	2.59	2.54	...	...
Dicyclohexylamine	222	...	U.T.T.	Water	67.7	68.0	7.58	7.75	...	...
Di-*n*-decylamine	70–1	...	U.T.T.	Acetone	...	...	...	...	5.73	5.76
Diethylamine	183	...	U.T.T.	Water	...	...	...	...	...	...
p-Diethylaminobenzaldehyde	113–14	368	375	Acetone	65.2	65.0	5.47	5.72	...	...
Diisoamylamine	189–91	...	U.T.T.	Acetone	...	...	...	...	...	...
Di-2-ethylhexylamine	147	...	U.T.T.	Acetone	69.4	69.1	9.32	9.30	...	...
Di-*n*-hexylamine	113–14	...	U.T.T.	Acetone	67.0	67.2	8.57	8.80	...	...
Dimethylamine	210	...	U.T.T.	Water	...	...	...	...	...	...
N,N-Dimethylaniline	133	312	316	Water	...	...	...	...	...	...
Di-*n*-nonylamine	92	...	U.T.T.	Acetone	...	...	...	...	6.08	5.88
Ethylamine	205–6	...	U.T.T.	Water	...	...	...	...	...	...
N-Ethylaniline	174–5	312	305	Acetone	...	...	...	...	...	...
Ethylenediamine	208	...	U.T.T.	Acetone	...	...	...	...	...	...
Ethyl nicotinate	127	342	339	Acetone	59.7	59.4	4.12	4.10	...	...
N-Ethyl-*o*-toluidine	179–82	326	329	Acetone	...	...	...	...	...	...
Glycine	...	133	253	Water	...	...	...	...	...	...
Guanidine	258	...	U.T.T.	Water	...	...	...	...	...	...
Guanine	...	342	337	Water	49.1	48.8	2.95	3.03	...	...
Histidine	...	269	263	Water	...	...	...	...	...	...
Hydrazine	...	223	227	Water	48.4	48.7	4.07	4.10	...	...
1-Hydroxyproline	...	322	311	Water	52.2	51.8	4.38	4.77	...	...
Isoquinoline	177	320	312	Acetone	...	...	...	...	...	...
2-Methoxy-6,9-dichloroacridine	138	469	460	Acetone	58.9	58.9–	3.01	3.20	...	...
5-Methoxy-8-nitroquinoline	158	396	400	Acetone	57.7	57.3	3.31	3.47	...	...
Methylamine	194–9	...	U.T.T.	Water	...	...	...	...	...	...
N-Methylaniline	174–6	298	290	Acetone	...	...	...	...	...	...
Methyl anthranilate	150–1	342	337	Acetone	59.7	59.8	4.14	4.24	...	...
5-Methyl-8-nitroquinoline	159	379	371	Acetone	60.2	59.9	3.45	3.56	...	...
6-Methylquinoline	156–8	334	332	Acetone	68.3	68.1	4.22	4.22	...	...
7-Methylquinoline	171	334	330	Acetone	...	...	...	...	8.38	8.74
α-Naphthylamine	207–10	334	335	Acetone	...	...	...	...	...	...
β-Naphthylamine	186	334	333	Acetone	...	...	...	...	...	...
Nicotinic acid	169	157	161	Acetone	57.4	57.2	3.21	3.66	...	...
m-Nitroaniline	182	...	U.T.T.	Acetone	...	...	...	...	...	...
o-Nitroaniline	143–5	329	328	Acetone	54.7	54.6	3.37	3.36	...	...
p-Nitroaniline	172	329	321	Acetone	...	...	...	...	...	...
5-Nitroquinoline	153–4	365	369	Acetone	59.2	58.9	3.04	3.21	...	...
8-Nitroquinoline	152–3	365	356	Acetone	59.2	58.9	3.04	3.16	...	...
m-Phenylenediamine (mono salt)	196–7	299	301	Water	60.2	59.9	4.38	4.54	...	...
p-Phenylenediamine (di salt)	246–8	245	246	Acetone	...	...	...	...	...	...
p-Phenetidine	191–3	328	331	Acetone	...	...	...	...	...	...
Phenylhydrazine	...	299	301	Alcohol	...	...	...	...	...	...
α-Picoline	147–8	284	278	Acetone	...	...	...	...	...	...
β-Picoline	141	284	281	Acetone	63.4	63.2	4.25	4.28	...	...
γ-Picoline	160–2	284	278	Acetone	63.4	62.9	4.25	4.23	...	...
Pipecolinic acid.1H_2O	133–4	338	334	Water	53.3	53.3	5.37	5.50	8.28	8.36
Piperidine	...	...	U.T.T.	Water	...	...	...	...	...	...
Pyridine	166–8	270	272	Acetone	...	...	...	...	...	...
4-Quinazolone	195	337	317	Acetone	60.5	60.3	3.29	3.43	12.45	12.43
Quinine.1H_2O (di salt)	210	...	...	...	63.0	62.5	5.01	5.06	7.72	7.79
Quinoline	148–9	320	325	Acetone	...	...	...	...	...	...
Semicarbazide	186–9	266	264	Acetone	45.1	45.0	3.79	4.01	...	...
Strychnine	226	525	521	Acetone	...	...	...	...	...	...
Sulfanilamide	203–5	363	364	Acetone	...	...	...	...	...	...
Theobromine	...	371	351	Water	51.8	52.1	3.53	3.87	...	...
o-Tolidine (di salt)	217–20	297	298	Acetone	...	...	...	...	...	...
m-Toluidine	184–6	298	296	Acetone	...	...	...	...	...	...
o-Toluidine	185–6	298	296	Acetone	...	...	...	...	...	...
p-Toluidine	183–4	298	294	Acetone	...	...	...	...	...	...
Urea.1H_2O	160	269	270	Water	44.6	44.6	4.12	4.26	15.60	15.85
2,4-Xylidine	184–7	312	312	Acetone	...	...	...	...	...	...

[a] All melting points in this column were taken with a melting point block and are corrected.
[b] U.T.T. unable to titrate.
[c] Compounds with no M.P. data gave indeterminate melting points.

A number of salts formed oils rather than crystalline derivatives: indandionates of 6-aminohexanol, 2,4-dimethylquinazoline, novocaine, and l-tyrosine.

15°, add 300 ml. of concentrated hydrochloric acid, and allow to stand for several hours; yield, 73%. (2-Nitro-1,3-indandione is commercially available from Krishell Laboratories, Portland, Ore.)

Preparation of Salts of 2-Nitro-1,3-indandione. Prepare a stock solution of the acid by dissolving 5 grams of 2-nitro-1,3-indandione in 150 ml. of acetone. Dissolve approximately 150 mg. of the test compound in a minimum amount of acetone, alcohol, or water and then add it to a slight excess of the stock solution. Place salts that do not crystallize immediately in a refrigerator for several hours, then filter, wash, and recrystallize from ethanol, water, or aqueous alcohol. The salts thus prepared have fairly sharp melting points (usually with decomposition).

The neutralization equivalents were determined according to the directions of Niederl and Niederl (*2*), using a phenolphthalein indicator. The data obtained from the titration of some of the compounds described by Wanag, together with those synthesized in this laboratory, are listed in Table I.

SUMMARY

The salt formation of 2-nitro-1,3-indandione as described by Wanag has been confirmed; over 40 new salts have been prepared and the titration characteristics of approximately 100 2-nitro-1,3-indandionates have been studied. 2-Nitro-1,3-indandione formed well defined salts with a wide variety of compounds, including the simple and substituted amines, alkaloids, nitrogen heterocycles, amino acids, amides, and halo, azo, and nitroamines. Most of the salts were colored; a few contained water of crystallization. Fairly accurate neutralization equivalents may be determined for the salts of weak and moderately weak bases such as quinoline or aniline but not for the stronger bases such as mono-, di-, and trialkyl amines, morpholine, and piperidine.

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Molecular Still for Small Volumes

JOHN N. ROPER, JR., *Tennessee Eastman Corporation, Kingsport, Tenn.*

THE semimicro molecular still described by Breger (*1*) is similar in principle to one with which the author is familiar. The still is simple to make and was designed to accommodate materials either too viscous or too small in volume to be distilled in any of the moving-film type stills now commercially available.

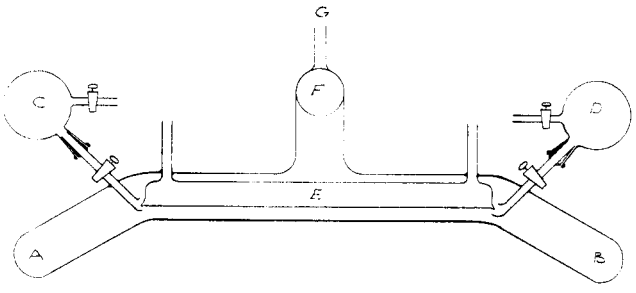

Figure 1. Molecular Still

Whereas the smallest sample distilled to date was a 14.5-gram mixture of lubricant oils which was divided into seven 2-gram cuts, smaller samples could be handled, and, if necessary, a model of smaller dimensions could be built.

CONSTRUCTION

The still is constructed of 26-mm. outside diameter Pyrex tubing having an over-all length of 380 mm. The internal water or compressed air–cooled condenser, *E*, is 200 × 14 mm. in outside diameter. The still is heated by means of a Nichrome wire heating coil wound on the outside of the still between the receiver tubes, and the current is regulated by a variable-voltage transformer. Data for a temperature-voltage calibration curve were taken before the still was set up for operation. The still is evacuated through a 29/42 standard-taper ground-glass joint which is in the line perpendicular to the plane of the drawing at *F*, allowing the still to rotate about *F* in the plane of the drawing. The vacuum is measured by a McLeod gage connected to the 8-mm. tube, *G*, by new rubber pressure tubing.

It has been observed that a stream of oil flowing over a hot surface has a strong tendency to occupy as small an area as possible in spite of efforts to spread it out. Therefore, as satisfactory results were obtained with the simple form of the still shown, various ideas for altering the evaporation surface to cause spreading of the distilland into a thinner film have been rejected.

OPERATION

The material to be distilled is poured through one of the receiver tubes into either end, *A* or *B*, of the still. Vacuum is then applied to the still to effect a preliminary degassing, after which the still is tipped to cause the distilland to flow slowly along the surface below the condenser, *E*, to the opposite end. Next, the still is tipped to cause the distilland to flow slowly back to its original position. The rocking of the still is continued while the distilland is thoroughly degassed as indicated by the reduction of the pressure to about 1 micron. Most molecular distillations can be carried out at an absolute pressure of from 1 to 5 microns. If any trouble is experienced in obtaining this vacuum with a reasonably high pumping capacity, all rubber connections in the system must be made with new rubber, the outer surface of which is treated with a good high-vacuum grease such as the silicone grease marketed by Dow-Corning. Then, while rocking is continued, the temperature is raised; this causes distillation to begin, which washes the spattered distilland from the condenser. The receiver-tube stopcocks are kept closed, after the initial evacuation of receivers *C* and *D*, during both the degassing and cleansing operations. During the actual distillation operation, the rocking of the still is necessarily intermittent, because time is required to allow the distillate to drain from the condenser through the receiver tubes into the receivers. The receiver-tube stopcocks are used to keep the distillate from flowing back from the receivers into the still when it is rocked.

By use of an auxiliary vacuum source, it is possible to take cuts without releasing the vacuum on the still. The receiver-tube stopcock is closed and the vacuum released on the receiver, the distillate is poured out, vacuum is again applied to the distillate receiver from the auxiliary source, and finally the receiver-tube stopcock is reopened.

ACKNOWLEDGMENT

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