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Gas Chromatographic Analysis of Pyridine Carboxylic Acids as their Esters. Comparison of Esterification Techniques

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A number of different esterification techniques are quantitatively investigated by internal standard technique and 2-methylpyridine-5-carboxylic acid, nicotinic, isonicotinic, and picolinic acid are separated by using Marlophen 87 and Carbowax as stationary phases. Nicotinic and isonicotinic acid are quantitatively esterified over acyl chloride hydrochlorides or by refluxing with acid catalyst and methanol in the presence of dimethoxypropane. Also 2-methylpyridine-5-carboxylic acid is quantitatively methylated by using the latter technique. 2-Methylpyridine-5-carboxylic acid, picolinic acid, and isocinchomeronic acid are quantitatively converted into their ethyl esters by refluxing with ethyl iodide and silver oxide in xylene. Applications are described for the analysis of products formed by the industrial oxidation of some alkyl pyridines with dilute nitric acid at high temperature and pressure.

By the oxidation of alkyl pyridines to carboxylic acids various products can be obtained, depending on the nature and position of the alkyl groups, oxidation agent, temperature, pressure, and reaction time. With some exceptions, the ease of attack seems to increase with the alkyl group position in the order $4 < 2 < 3$ [1] (see also Ref. 2). A carboxyl group in position 2 can be decarboxylated much easier than those in positions 3 and 4.[3] Much work remains to be done on the study of partial oxidation of polyalkyl pyridines.

The purpose of this work was to develop methods for the quantitative determination of pyridine carboxylic acids in mother liquors and crude samples obtained by the industrial oxidation with dilute nitric acid at high temperature and pressure of 2-methyl-5-ethylpyridine and of commercial mixtures of pyridine homologues (2,4-lutidine fraction).[4] The oxidation of 2-methyl-5-ethylpyridine mainly proceeds over 2-methylnicotinic acid to isocinchomeronic acid, which can be decarboxylated to nicotinic acid, and the crude nicotinic or isocinchomeronic acid may therefore contain certain amounts of the

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other acids. Technical grade isonicotinic acid prepared by the oxidation of the 2,4-lutidine fraction in the above way contains a few per cent nicotinic acid. Investigations were also conducted on the determination of picolinic acid in mixtures of other pyridine carboxylic acids, although because of the ease of decarboxylation of the 2-carboxyl group it is not found in the above samples. With the exception of the determination of isocinchomeronic acid no satisfactory methods of analysis of these mixtures were found in literature. The results obtained in this work appear to show that the methods described may easily be applied also to the detection and determination of small amounts of pyridine carboxylic acids in other kinds of samples.

As a member of the vitamin B complex, nicotinic acid has been thoroughly investigated, and many methods, mainly microbiological (see for instance Refs. 5 and 6) and colorimetric (see for instance Refs. 7 and 8), have been devised for its determination. Herington [9] determined isonicotinic acid colorimetrically in the presence of nicotinic and dipicolinic acid after reaction with trisodium pentacyanoammino ferrate. However, the max. error was about 4 % in the 10–100 % range, and nicotinic acid was reported to have a negative influence. The method described by Cheng and Riddick [10] (see also Refs. 11 and 12) for the colorimetric determination of isocinchomeronic acid with Fe^{3+} in the presence of nicotinic acid, 2-methyl-5-ethylpyridine, and nitric acid has been used in the laboratory at Bofors with good results. The paper chromatographic separation and detection of pyridine mono- and dicarboxylic acids have been reported [13,14] (see also Refs. 15–20), but nothing was mentioned about quantitative determination. Böddeker and Mishkin [21] determined nicotinic acid in coffee by paper chromatography. Studies on nicotinic acid by means of paper electrophoresis,[22] thin layer chromatography,[23,24] and ion exchange [25,27] have been reported, but no separation from the other isomers was investigated. The pyridine carboxylic acids can be determined by means of polarography, but the separation is difficult to accomplish by ordinary techniques [28,29] (see also Refs. 30–32).

Prior to GLC it is advisable to convert the pyridine carboxylic acids into less polar derivatives of higher vapor pressure. The free acids, containing both the pyridine nitrogen and a carboxyl group, may otherwise dimerize and cause distorted peaks. No investigations have been published about the methylation of pyridine carboxylic acids for gas chromatography. In the present work a quantitative investigation has been made of the esterification techniques summarized in Table 1.

Kuhn and Brydówna [33] reported an 89 % yield of ester by treating nicotinic acid with diazomethane in moist ether, and Mariella and Havlik [34] obtained a 78 % yield of 2-methyl-6-hydroxypyridine-5-carboxylic acid methyl ester by distilling diazomethane-ether into a suspension of the acid in methanol. The methylation of fatty acids for GLC by adding methyl iodide to the silver salt of the acid has been reported to give quantitative yields.[35] Fürst and Jelesaroff [36] have prepared ethyl picolinate in quantitative yield by refluxing the acid and Ag_2O with ethyl iodide in xylene. The preparation of the acyl chlorides of pyridine monocarboxylic acids and isocinchomeronic acid by refluxing with thionyl chloride has been investigated [37–42] and the yield is reported to be quantitative for nicotinic and isonicotinic acid. The acyl chlorides were said to react instantaneously with methanol to give the methyl esters. Klosa [43] used phosphorus oxychloride as an acid catalyst when refluxing picolinic acid with methanol and reported 90–95 % yield of ester. Gee [44] methylated prior to GC, among other products, pyrrolidone carboxylic acid in high yield by using a combination of thionyl chloride and hydrochloric acid as a catalyst. Boiling fatty acids in BF_3-methanol for 2–3 min. gave about 80 % yield of esters for gas chromatography as reported by Metcalfe and Schmitz.[45] The use of 2,2-dimethoxypropane as a dehydrating agent has been employed for esterification by several authors (see for instance Refs. 46–51), but no investigation has been published on pyridine carboxylic acids with this method. A summary of the recoveries obtained by the author when using the methods investigated in this work is presented in Table 1.

Table 1. Comparison of esterification techniques of pyridine carboxylic acids.[a] For details see the text.

Esterification technique	Nicotinic acid	Isonicotinic acid	Methyl nicotinic acid	Picolinic acid	Isocinchomeronic acid
Diazomethane	+	+	+	+	
Silver salt + methyl iodide according to Ref. 35	−				
Acid, Ag_2O, and methyl iodide	−	−	+	+	
Acid, Ag_2O, and ethyl iodide	+	+	Q	Q	Q
Via acyl chloride hydrochloride	Q	Q	−	+	
$POCl_3$-methanol				−	
$HCl \cdot SOCl_2 \cdot$ methanol	+	−	−	−	
$BF_3 \cdot$ methanol	−				
DMP · methanol · acid catalyst	Q	Q	Q	−	

[a] Q = quantitative yield (more than 95 %). + = over 60 % yield. − = under 60 % yield.

EXPERIMENTAL

Materials. Pyridine carboxylic acids and isocinchomeronic acid diethyl ester used in this work were recrystallized several times and the pyridine monocarboxylic acid esters were subsequently purified by fractional distillation under reduced pressure. The purity of the pyridine carboxylic acids and esters was determined to be more than 99 % by potentiometric titration with perchloric acid in acetic acid, and the pyridine monocarboxylic acids and esters were shown to be free from the other isomers by means of gas chromatography. The melting point of isocinchomeronic acid diethyl ester was found to be 46−46.5°C, and the free acid melted at 249.5°C under decomposition.

Benzene, *p*-xylene and ether were dried over metallic sodium, and moist ether was obtained by saturating with water at 20°. Thionyl chloride and 2,2-dimethoxypropane were purified by distillation under reduced pressure. HCl-methanol, prepared by passing dry HCl through methanol, was stored in small injection bottles, and the acidity was checked by titration before use. BF_3-methanol was freshly prepared by passing BF_3 into methanol, and the BF_3 content was determined by the increase in weight of the solution. The phosphorus oxychloride (Riedel De Haën AG, Hannover) was used without further purification and the methanol (E. Merck AG, Darmstadt) used was of *p.a.* quality with a water content (according to K. Fischer) of less than 0.05 %. Ethyl iodide (BDH), methyl iodide (Fluka), and silver oxide were taken from freshly opened ampoules and bottles without further purification. Internal standards were distilled under reduced pressure.

Apparatus. Gas chromatograph: F & M, model 720 with W2 four-filament detector. Recorder: 1 mV Honeywell-Brown. Chart width 12″. Carrier gas: H_2. Injection port temperature: 210°C.

Columns: Isocinchomeronic acid diethyl ester was analyzed at 150°C on a 1 m × ¼″ glass column filled with 0.75 % Marlophen 87 (Chemische Werke Hüls AG) on Chromosorb G, 70−80 mesh (Johns Manville) and the separation of small amounts of nicotinic acid in isonicotinic acid was performed at 100°C on a 2 m × ¼″ column filled with 4 % Carbowax 400 on Chromosorb G. All the other investigations were performed at 165° on a 1 m × ¼″ column filled with 20 % Marlophen 87 on Chromosorb W. Chromatograms are presented in Figs. 1−4.

Preparation of esterified samples for GC, calculation. Neutral samples were injected for GC after addition of internal standard and correction of the volume. It was necessary

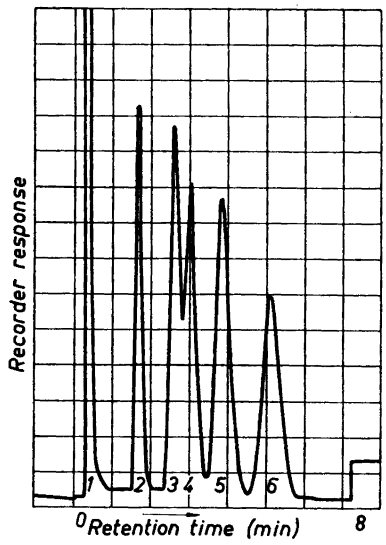

Fig. 1. Mixture of methyl esters of pyridine carboxylic acids. (20 % Marlophen 87 on Chromosorb W, 165°C.)
1. Solvent. 2. N,N-dimethyl aniline (internal standard). 3. isonicotinic acid. 4. nicotinic acid. 5. 2-methylnicotinic acid. 6. picolinic acid.

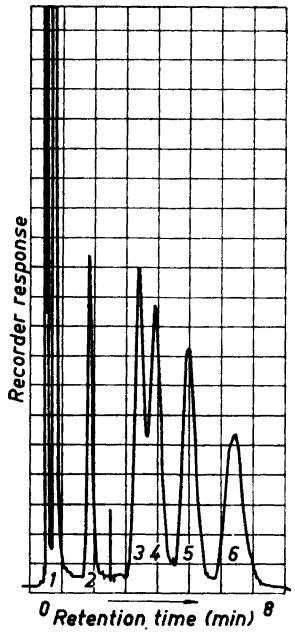

Fig. 2. Mixture of ethyl esters of pyridine carboxylic acids. Exactly the same conditions as in Fig. 1.
1. solvent. 2. N,N-dimethyl aniline (internal standard). 3. isonicotinic acid. 4. nicotinic acid. 5. 2-methyl nicotinic acid. 6. picolinic acid.

to neutralize acid samples because of the basic pyridine nitrogen, and this was performed by adding solid ammonium carbonate to the solution until no further carbon dioxide was evolved. After addition of internal standard and centrifugation, the liquid phase was decanted and made up to volume.

Attempts were made to quantitatively split pyridine hydronitrates and hydrochlorides to the free bases, but this required injection temperatures of more than 350°C, and was therefore not attempted for the pyridine carboxylic acids. The usual method of adding water to the acid esterification mixture, neutralizing and extracting with ether gave erroneous results, mainly because of the volatility of the free carboxylic acid esters.

The amount of ester obtained was determined against an internal standard in the usual way. The internal standard chosen, although not quite ideal with regard to the inertness (it was later replaced by octanol-1), was N,N-dimethyl aniline. Factors converting area-% to weight-% according to the formula below were determined by running freshly prepared test mixtures of internal standard and ester at the same conditions as and alternately with the samples.

$$f = \frac{Y_{\text{I.S.}} \times W_{\text{ester}}}{W_{\text{I.S.}} \times Y_{\text{ester}}}$$

where $Y_{\text{I.S.}}$ and Y_{ester} are the areas under the peaks of the internal standard and ester respectively and $W_{\text{I.S.}}$ and W_{ester} are the weights or percentages of the internal standard and ester in the test mixture.

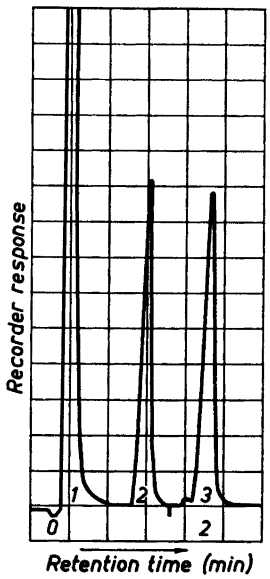

Fig. 3. Isocinchomeronic acid diethyl ester. (0.75 % Marlophen 87 on Chromosorb G, 150°C.)
1. solvent. 2. 2,6-Dinitrotoluene (internal standard). 3. isocinchomeronic acid.

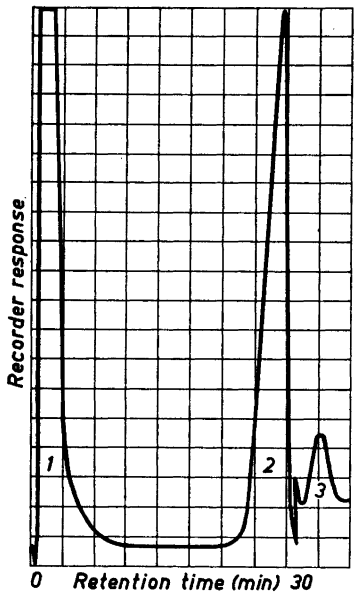

Fig. 4. 1 % nicotinic acid in isonicotinic acid as methyl esters. (4 % Carbowax 400 on Chromosorb G, 100°C.)
1. solvent. 2. isonicotinic acid (Sensitivity 1/8). 3. nicotinic acid (sensitivity 1).

The factors were found to depend on the retention time as shown in Table 2, and therefore factors for nicotinic acid ethyl ester and picolinic acid methyl ester were assumed to have values intermediate to the others (no quantitative esterification technique for these esters is described in this work). Internal standard for isocinchomeronic acid diethyl ester was 2,6-dinitrotoluene and the factor was determined to be 1.00.

Esterification techniques

1. *Diazomethane.* Diazomethane ether prepared from *p*-tolylsulfomethyl nitrosamide [52] and KOH is slowly distilled into a suspension or solution of 0.05 g acid in a mixture of

Table 2. Conversion factors of pyridine carboxylic acid esters relative to N,N-dimethyl aniline.

	Methyl ester	Ethyl ester
Isonicotinic acid	1.11	1.12
Nicotinic acid	1.11	
Methylnicotinic acid	1.20	1.19
Picolinic acid		1.37

1 ml methanol and 1 ml ether until no further nitrogen is evolved and the solution has turned yellow. After standing without a cover for 1 h, the volume is adjusted, internal standard added and the sample injected for GC.

At different times, three investigations were made and the results are presented in Table 3. Methanol and dry or moist ether were found to give significantly lower yields

Table 3. Yield of ester by using the diazomethane method. Mean values of two or more GC-analyses of the same methylation mixture.

Investigation number	Yield (%)			
	Nicotinic acid	Isonicotinic acid	Methylnicotinic acid	Picolinic acid
1 (0°C)	95.4 91.4			
1 (20°C)	94.6 91.6 88.0 93.3 86.0 94.0			
2 (−10°C)	75	60		40
3 (20°C)	70	75	80	52

and especially the ether solutions turned dark brown. As appears from the table, the recovery values from the first investigation were not reproduced, and the procedure above was considered unsuitable for quantitative analysis. Because of the disadvantage in handling diazomethane due to toxicity and explosion hazard, no further studies were performed with this technique.

2. Silver salt and methyl iodide. The macromethod described in Ref. 35 was used for nicotinic acid: Preparation of the silver salt by addition of $AgNO_3$ to the alkalized solution of the acid, removal of solvent and addition of methyl iodide. After standing overnight, the solution was analyzed by GC and the yield was found to be about 5 %. An explanation of this low yield may be the formation of nicotinic acid N-methyl betaine in the presence of potassium ions,[53] here used in the neutralization of nicotinic acid.

3. Refluxing with Ag_2O and alkyl iodide. 0.5 g acid (0.25 g isocinchomeronic acid) is mixed with 1 g Ag_2O. 5 ml dried *p*-xylene and 1.3 g ethyl iodide is added and the mixture is refluxed for 1 h. After cooling, the liquid phase is decanted, the residue is washed with xylene, the volume is adjusted, internal standard is added and the sample is injected for GC.

Investigations were also made using methyl iodide and benzene instead of ethyl iodide and xylene.

The results are shown in Table 4, and as can be seen the recovery of ethyl esters is excellent for methylnicotinic acid, picolinic acid, and isocinchomeronic acid, but lower for the other acids. The yields of methyl esters were lower than those of ethyl esters. A small peak appears immediately before the isocinchomeronic acid diethyl ester on the chromatogram (Fig. 3) and may be a by-product formed during the esterification. It is not formed when preparing the diethyl ester over the acyl chloride according to Ref. 41. but it did show some variance with the quality of the isocinchomeronic acid.

Table 4. Yields of esters using the Ag_2O + alkyl iodide method. Mean values of two or more GC-analyses of the same esterification mixture.

Alkyl iodide used	Yield (%)				
	Nicotinic acid	Iso-nicotinic acid	Methyl-nicotinic acid	Picolinic acid	Isochincho-meronic acid
Methyl iodide	44 56	15 20	70	60 70	
Ethyl iodide	60	60	99.1	101.0 99.3	98.9

4. Esterification via acyl chloride hydrochloride. 2.5 g acid is refluxed for 1 h with 10 ml purified thionyl chloride, the excess thionyl chloride is distilled under reduced pressure and the residual acyl chloride hydrochloride is refluxed with methanol for 1 h. After neutralization, internal standard is added and the solution is injected for GC.

The yields are presented in Table 5. Prior to observing the risk of side-reactions at higher temperatures, no esters were obtained from picolinic and methylnicotinic acid, and these solutions turned dark. As will be seen from the table, the recoveries are quantitative for isonicotinic and nicotinic acid, and the reproducibility is good (see also Table 8). The yield of picolinic acid ester was about 80 % and that of methylnicotinic acid ester about 20 %.

5. Methanol and acid catalyst (no dimethoxypropane present). a) 7 ml methanol, 0.5 g acid, and 0.05 g $POCl_3$ is refluxed for 2.5 h. b) 0.05 g acid, 0.1 ml $SOCl_2$, and 10 ml HCl-methanol (8.5 %) is refluxed for 10 min. c) 0.1 g acid in 15 ml BF_3-methanol (125 g BF_3/l) is boiled for 2 or 5 min. The acid solutions are neutralized, internal standard is added, and the sample is injected for GC.

The results are shown in Table 6 and as can be seen the recoveries are low. Modifications may make it possible to obtain higher yields, but this was not investigated.

6. Dimethoxypropane (DMP) as dehydrating agent. a) 40 mg acid, 0.3 ml DMP, and 5 ml HCl-methanol (7.5 %) is sealed in an ampoule and allowed to stand at 60° overnight. For the first hour, the solution was shaken occasionally until the acid had gone into

Table 5. Recovery of methyl esters *via* acyl chloride hydrochloride. Figures in brackets represent repeated GC-analysis of the same methylation mixture.

Acid	Yield %	Acid	Yield %
Nicotinic acid	100.0 (100.2 97.6) 99.4 96.3 (100.5 99.2) 99.2 98.9 (103.1 100.0) 97.0	Isonicotinic acid	97.4 (95.2 100.1) 99.5 95.6 (102.1 98.1) 98.6 79 21

Table 6. Recovery of methyl esters with alcohol and acid catalyst.

Method	Yield (%)			
	Nicotinic acid	Iso-nicotinic acid	Methyl-nicotinic acid	Picolinic acid
$POCl_3$ · methanol				8
$SOCl_2$ · HCl · methanol	64	49	29	7
BF_3 · methanol	10			

solution. b) 0.1 g acid, 0.6 ml DMP, 10 ml methanol, and 0.6 ml conc. H_2SO_4 is refluxed for 4 h. The solutions are neutralized, internal standard is added, and the sample is injected for GC.

During the reaction, the solutions turned light yellow but no interfering peaks from the dimethoxy propane were obtained on the chromatograms. Addition of dimethyl sulfoxide did not affect this colour. The recoveries are presented in Table 7 (see also Tables 9 and 10). Attempts to esterify at room temperature with various proportions of reagents gave a yield of only a few per cent. As appears from Table 7, the recovery is quantitative for nicotinic, isonicotinic, and methylnicotinic acid, but only about 50 % for picolinic acid.

APPLICATIONS AND DISCUSSION

No method was found which quantitatively esterified all the pyridine carboxylic acids under investigation in this work, but there is at least one

Table 7. Recovery of methyl esters with alcohol, acid catalyst and dimethoxypropane. Figures in brackets represent repeated GC-analysis of the same methylation mixture.

Method	Yield (%)			
	Nicotinic acid	Isonicotinic acid	Methylnicotinic acid	Picolinic acid
HCl as catalyst at 60° in ampoule	98.6 100.5 101.1 99.3 99.3 101.1 99.8 99.3 99.5	96.2 97.3 94.8	97.8 100.3 101.6 96.9	
H_2SO_4 as catalyst, refluxed 3 h	92.1		98.8	
H_2SO_4 as catalyst, refluxed 4 h	99.2		99.8	50
H_2SO_4 as catalyst, refluxed 5 h	100.2		98.0	

quantitative method for each of the acids. The esterification techniques can easily be applied to the analysis of pyridine carboxylic acids in a wide variety of samples. For example, the ampoule esterification in the presence of DMP can be used for the determination of very small amounts of nicotinic acid in biological materials by using smaller amounts of reagents and a more sensitive gas chromatographic detector. At the Bofors laboratory the methods described below have been used in the routine control of mother liquors and crude samples of pyridine carboxylic acids.

1. *Determination of nicotinic acid (0—4 %) in isonicotinic acid:* The methylation over acyl chloride hydrochlorides was chosen because a more concentrated solution was required, otherwise too small amounts of the nicotinic acid were injected. The procedure is described under "experimental". Test mixtures were prepared and the factor for nicotinic acid was determined according to the formula

$$f = \frac{Y_{INS} \times W_{NI}}{Y_{NI} \times W_{INS}},$$

where Y_{INS} and Y_{NI} are the areas under the isonicotinic and nicotinic acid peaks respectively, and W_{INS} and W_{NI} are the weights and percentages of nicotinic acid and isonicotinic acid in the test mixtures. The isomeric proportions were determined by internal normalization in the usual way. As will be seen from Table 8, the linearity is good and the precentage of nicotinic acid in isonicotinic acid can be determined within ± 0.1 %.

2. *Determination of nicotinic acid and methylnicotinic acid in isocinchomeronic acid:* the method chosen was refluxing with methanol and sulfuric acid in the presence of dimethoxypropane because this was the only method

Table 8. Factors for nicotinic acid in isonicotinic acid obtained by repeated gas chromatographic analysis of 4 esterification mixtures.

Sample number	Area-% nicotinic acid	Factor
1	2.3	0.87
1	2.2	0.91
1	2.4	0.83
1	2.3	0.87
2	2.1	0.96
2	2.1	0.96
2	2.3	0.87
2	2.2	0.91
3	1.4	0.92
3	1.3	1.04
4	5.3	0.99
4	5.5	0.96
	Mean value:	0.92

Table 9. Determination of small amounts of methylnicotinic and nicotinic acid in isocinchomeronic acid.

Added (g)			Found (g)		Correction factor	
Nicotinic acid	Methyl-nicotinic acid	Isocincho-meronic acid	Nicotinic acid	Methyl-nicotinic acid	Nicotinic acid	Methyl-nicotinic acid
0.0059	0.0050	2	0.0054	0.0030	1.1	1.7
0.0050	0.0043	3	0.0038	0.0022	1.3	1.9
0.0033	0.0020	1	0.0030	0.0011	1.1	1.8
0.0032	0.0013	1	0.0036	0.0007	0.9	1.9
0.0050	0.0099	1	0.0042	0.0055	1.2	1.8
			Mean value		1.1	1.8

which quantitatively converted both nicotinic and methylnicotinic acid to their methyl esters. It is possible to determine percentages if these acids as low as 0.1 %, but calibration factors are needed because of the influence of isocinchomeronic acid. The sample, which must not contain more than about 0.01 g of nicotinic and methylnicotinic acid, is refluxed for 4 h with 5 ml methanol, 0.3 ml dimethoxypropane and 0.3 ml conc. sulfuric acid. To the cold mixture, octanol-1 is added as internal standard, the suspension is thoroughly mixed and part of the liquid phase is collected. After neutralization and centrifuging it is injected for GC. Recovery values and conversion factors are presented in Table 9. No deterioration of the column was found after having analyzed more than thirty samples in series.

3. *Determination of nicotinic, isonicotinic, and methylnicotinic acid in mother liquors containing alkyl pyridines and dilute nitric acid.* The sample

Table 10. Determination of pyridine carboxylic acids in dry and aqueous mixtures containing nitrate.

Added (g)					Found (g)		
Nico-tinic acid	Iso-nicotinic acid	Methyl-nicotinic acid	Isocincho-meronic acid	$NaNO_3$	Nico-tinic acid	Iso-nicotinic acid	Methyl-nicotinic acid
0.102		0.102		0.8			0.099
	0.102			0.4	0.097		
				0.4		0.101	
0.044		0.054	0.03	[a]	0.044		0.052
0.030		0.061	0.02	[a]	0.029		0.060
0.036		0.051	0.02	[a]	0.037		0.048

[a] 2 ml 4 N HNO_3, 0.02 g 2-methyl-5-ethylpyridine, and 3 ml H_2O added, the solution neutralized and evaporated to dryness.

containing not more than 0.1 g of pyridine carboxylic acids is neutralized with sodium hydroxide to about pH 8 and evaporated to dryness, the last amounts on a steam-bath and in an oven at 140°. The pyridine carboxylic acids are esterified by refluxing with 10 ml methanol, 0.8 ml conc. H_2SO_4, and 0.8 ml dimethoxypropane, and the percentages are determined by gas chromatography using octanol-1 as internal standard.

The recoveries of synthetic mixtures are presented in Table 10. The nitrate does not interfere during the esterification and is removed to a large extent because of its slight solubility in methanol. Less hydrophilic internal standards such as 2,6-dichlorotoluene gave too high results, probably because of the salting out effect by the nitrate. N,N-Dimethyl aniline was not suitable because of decomposition in the presence of nitrate.

Picolinic acid, if present, is easily detected on the chromatogram and a semi-quantitative value of its percentage in the sample can be obtained.

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