Occurrence of Pyridines and Other Bases in Orange Oil

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Cold-pressed Florida (Valencia) orange oil contains a series of bases, the main one of which is 3-hexylpyridine at ca. 20 ppb. Smaller amounts of 3-heptyl-, 3-octyl-, and 5-hexyl-2-methylpyridine are also present, as are 3-(4-methylpentyl)- and 3-(4-methylhexyl)pyridine. There are traces of other, more generally known, pyridines. 3-Hexyl-, 3-heptyl-, and 3-octylpyridine and 5-hexyl-2-methylpyridine were also detected in Brazilian (Pera) orange oil. The flavor threshold concentration of 3-hexylpyridine in water is 0.28 ppb.

INTRODUCTION

In spite of the great number of analyses undertaken on cold-pressed Valencia orange oil, there has been no systematic study of the bases. Methyl anthranilate, methyl *N*-methylanthranilate, and diphenylamine have been detected in sweet orange peel oil and methyl anthranilate and diphenylamine in bitter orange peel oil (Maarse and Visscher, 1989). Diphenylamine (A referee suggested that the presence of diphenylamine could be the result of contamination by pesticides.) has also been identified in bitter orange juice (Maarse and Visscher, 1989) and in lemon and grapefruit peel oil (Piorr and Tóth, 1967; Karawya et al., 1984). Indole has been found in satsuma mandarin (*Citrus unshiu*) peel oil (Yajima et al., 1979), and 3-phenylpyridine is reported (without experimental detail) to occur in *C. unshiu* and *Citrus natsudaidai* (Sakurai et al., 1983). Traces very often add special notes to flavors, and that is well-known with pyrazines, which are among the most potent flavor constituents [see, for example, Buttery et al. (1969a,b)]. Therefore, it seemed interesting to look in the direction of the bases present in small amount in the oils. We now give the result of this search in cold-pressed oil of sweet oranges (*Citrus sinensis* L.) from Brazil (Pera) and Florida (Valencia) oranges.

EXPERIMENTAL PROCEDURES

General Procedures. ^1H NMR (360 MHz) and ^{13}C NMR (90.5 MHz) spectra were measured on a Bruker WH-360 instrument modified to an AM model and interfaced to an Aspect 2000 computer or on a Bruker AMX 360 instrument. Mass spectra were obtained using a Finnigan 1020 quadrupole spectrometer coupled with a gas chromatograph containing a 60-m glass capillary column coated either with Supelcowax (polar) or SPB-1 (apolar) stationary phase.

Origin of the Oil. Cold-pressed orange oil is expressed from the peel of fresh oranges according to the Food Machinery Corp. (FMC) procedure. The Brazilian oil was purchased from Citrusuco and the Florida oil from Intercit Inc.

Extraction of Orange Oil. *Preliminary Qualitative Examination.* A mixture of orange oils from Brazil and Florida was treated in a large-scale process involving extraction with aqueous ethanol to separate most of the hydrocarbons (deterpenation), the material from the polar phase being distilled at 0.5-mm pressure. The top fraction (up to bp 66 °C) consists mostly of residual limonene and other monoterpenes, and the fraction with bp 66–143 °C was our raw material, representing about 1.8% of the original oil. This material was conventionally extracted with sulfuric acid (10%), basified, and then extracted with pentane of high purity. Evaporation of the pentane yielded a mixture containing the bases.

Quantitative Examination. We extracted 100 kg each of untreated cold-pressed orange oil from Brazil and Florida using a 250-L stainless steel reactor. Prior to this, the reactor was scrupulously cleaned by washing for 1 day with sulfuric acid (15%), washing with water, then steaming for 2 days and drying. The orange oil (100 kg) was stirred with sulfuric acid (10%, 2 × 1000 mL) and then with water (2 × 1000 mL). Each time the mixture was stirred for 4 min and allowed to decant for 1–2 h. The combined extracts were clarified by filtration through Hyflo Supercel, and the aqueous acid part was extracted with high-purity pentane (3 × 500 mL) and then made basic with solid sodium carbonate. The basified solution was extracted with pentane (3 × 200 mL), dried ($MgSO_4$), and concentrated (40 °C, 100 mbar) to afford 257 mg of material from the Brazilian oil and 280 mg from the Florida oil. The bases of the two sources were accompanied by different products. The Florida oil has given some emulsion during the extraction, which was probably the reason that the portion extracted with acid still contained a considerable amount of limonene, while the cleanly separating Brazilian oil contained very little limonene in this fraction but larger amounts of methanediols. The extracts were subjected to a second treatment by dissolving them each in high-purity ethyl acetate (20 mL) and extracting the bases with sulfuric acid (10%, 2 × 7 mL) and water (2 × 7 mL). After the aqueous acid extracts were basified (solid sodium carbonate) and the bases were extracted with ethyl acetate (3 × 10 mL), workup yielded 28 mg of material from the Brazilian oil and 8 mg from the Florida oil.

Synthesis of Pyridines: The Tchitchibabine Method, 3-Hexylpyridine (1). A solution of sodium amide in liquid ammonia was prepared by adding small pieces of sodium to about 300 mL of liquid ammonia containing ferric nitrate (0.1 g) until a total of 5.78 g had been introduced. After reaction was complete, 3-picoline (3-methylpyridine, 20.48 g) was added at −30 °C (10 min), followed by pentyl bromide (29.8 mL, 36.24 g) over 15 min (this corresponds to 0.5 mol each of sodium and alkyl bromide and 0.45 mol of picoline). The mixture was stirred for a further 15 min, the ammonia was allowed to evaporate, and then ice water was added to complete the hydrolysis. The mixture was extracted with ether (twice) and the extract washed with water, and then the bases were removed in hydrochloric acid (4 × 100 mL, 10%), followed by one washing with water. The combined aqueous phases were basified (sodium hydroxide, 20%, 0 °C), and the pyridine was extracted into ether. Conventional workup yielded 23 g of crude product, which was distilled to yield 1: 17 g, bp 65–71 °C/0.1 mbar.

This method was used to make the following pyridines: (a) from 3-methylpyridine, 1–4, 6; (b) from 2-methylpyridine, 7, 8, 10; (c) from 4-methylpyridine, 11; (d) from the corresponding ethylpyridines, 5, 9, 12; (e) from 3,5-dimethylpyridine (with 1 equiv of the reactants), 16 (a small amount of 3,5-dihexylpyridine was separated by distillation).

Methylation of 3-Hexylpyridine [cf. Fraenkel et al. (1970), Lyle and Comins (1976), Comins and Abdullah (1982), and Comins and Mantlo (1983)]. A solution of 3-hexylpyridine (1,

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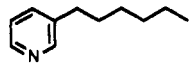
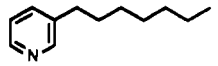
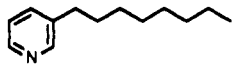
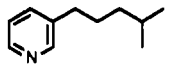
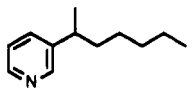
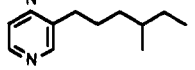
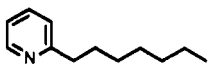
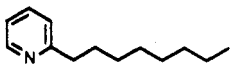
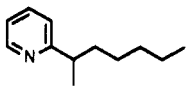
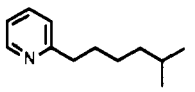
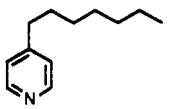
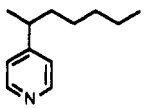
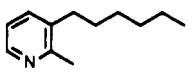
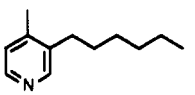
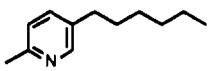
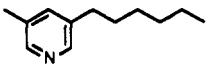
Table I. Mass Spectra of Substituted Pyridines

formula	structure	bp (0.1 mbar), °C	MW	$M^{\bullet+}$	$M-1^+$	162	148	134	120	107	106	93	92
	1[c]	65–67	163	10	10		3	9	33	14	85	100	70
	2[c]	72–74	177	6	4	2	6	13	15	15	75	100	40
	3[c]	108	191	5	11	8	14	7	18	19	98	100	40
	4[c]	53–55	163	23	9		12	2	7	30	40	100	72
	5	65	177	8	5	5	1	3	20	15	100	1	6
	6[c]	*b*	177	10	8	9	30	5	30	33	71	100	88
	7	139–141[a]	177	1	1	1	4	6	20	5	28	100	5
	8	118	191	1	1	2	4	5	19	5	27	100	5
	9	44–45	177	3	1	7	2	17	48	100	62	7	5
	10	37–44	177	1	1	5	0	10	20	7	30	100	5
	11	63–65	177	2	1	1	3	9	7	7	45	100	13
	12	61	177	22	3	4	6	2	15	100	95	3	8
	13	*b*	177	22	1	9	3	9	25	55	100	5	4
	14	*b*	177	2	4	11	3	10	55	88	100	5	3
	15[c]	*b*	177	10	5	2	4	15	30	35	100	3	2
	16	70–73	177	16	8	3	6	32	80	100	87	7	8

[a] At 10 mbar. [b] Distilled in bulb tube, oven temperature 100 °C. [c] Identical with natural material from orange oil.

3.31 g, 20.3 mM) and cuprous iodide (130 mg) in dry tetrahydrofuran (33 mL) was cooled to −22 °C. Ethyl chloroformate (1.29 mL, 13.4 mmol) was added, and the mixture was stirred at −20 °C for 15 min. A solution of methylmagnesium iodide (13.5 mmol in 11 mL of THF) was added dropwise. After stirring for a further 15 min at −20 °C, ammonium chloride (20%, aqueous) was added and the mixture was extracted with ether. The organic phase was washed twice with hydrochloric acid (10%) and then water, dried, and concentrated. Chromatography on silica gel gave 1-(ethoxycarbonyl)-3-hexyl-4-methyl-1,4-dihydropyridine (**25**), contaminated with a few percent of 3-hexyl-4-methylpyridine (**14**).[1] ^{1}H NMR spectrum of the dihydropyridine (**25**): 0.89 (3 H, t), 1.10 (3 H, d, J = 7 Hz), 1.9–2.1 (2 H, mult), 4.23 (2 H, q), 4.79 and 4.89 (1 H together, each 4 lines, C(4)H), 6.50 and 6.63 (together 1 H, each s, C(s)H), 6.69 and 6.80 (together 1 H, each d). The doubling of signals in this type of compound has been commented on by Fraenkel et al. (1970). MS: 251 ($M^{\bullet+}$, 2), 236 ($M-CH_3$, 25), 208 (11), 192 (33), 178 (5), 164 (100), 148 (5), 134 (22), 120 (43), 107 (72), 106 (80), 93 (45). Treatment of the dihydropyridine (**25**, 2.9 g) with chloranil (3.1 g, 12.6 mmol) at reflux under nitrogen for 8 h yielded 3-hexyl-4-methylpyridine (**14**), which was isolated by cooling and extracting in ether (50 mL) and sodium hydroxide (25 mL, 1 N). After 15 min, the mixture was filtered through Celite and the organic phase washed with brine. Drying and concentration yielded 0.4 g of nearly pure 3-hexyl-4-methylpyridine (**14**), which was purified by bulb distillation (MS given in Table I, ^{1}H NMR data in Table III).

When the reaction was carried out using 25.2 g of 3-hexylpyridine, 15.2 g of ethyl chloroformate, and a Grignard reagent prepared from 3.4 g of magnesium and 19.9 g of methyl iodide in dry THF, there were obtained three dihydropyridines, **26**, **24**, **25**, in the proportions 10:2:5, eluted in that order from the Supelcowax column. Chromatography on silica gel (order of elution **25**, **24**, **26**) enabled **24** and **26** to be purified sufficiently for spectral characterization. ^{1}H NMR of **24**: 0.88 (3 H, t), 1.11 (3 H, d) 4.72 and 4.82 (1 H together, each 5 lines, C(2)H), 5.55

Table II. Bases Identified in Florida Orange Oil

peak	compd	structure	retention indices: Supelcowax 60 m	retention indices: SPB-1 60 m
not shown	3-ethylpyridine		1409	939
1	3-propylpyridine		1494	1034
5	*N,N*-dimethylaniline		1574	1073
6	2-pentylpyridine	23	1592	1181
7	3-butylpyridine		1602	1138
8	2-methyl-5-(1-methylethenyl)pyridine	20	1610	1116
10	(*E*)-3-(but-1-enyl)pyridine	21	1657	1145
11	3-methyl-*N,N*-dimethylaniline (*N,N*-dimethyl-*m*-toluidine)		1664	1174
13	3-pentylpyridine		1706	1242
15	*N*-methylaniline		1746	1041
16	3-(4-methylpentyl)pyridine	4	1759	1303
20	3-hexylpyridine	1	1813	1350
21	5-hexyl-2-methylpyridine	15	1837	1410
22	ethyl 3-pyridinecarboxylate (ethyl nicotinate)		1844	1191
23	3-(4-methylhexyl)pyridine	6	1888	1418
26 (mixture)	5-acetyl-2-methylpyridine		1902	1162
28	3-heptylpyridine	2	1925	1455
40	3-octylpyridine	3	2034	1560
43	methyl *N*-methylanthranilate		2123	1388
55	methyl anthranilate		2291	1318
57	2-phenylpyridine	17	2299	1438
58	3-phenylpyridine	18	2306	1442
60	4-phenylpyridine	19	2327	1449
61	2-methyl-5-phenylpyridine	22	2332	1513
72	2-(methylamino)benzyl alcohol		2476	1323
not shown	2,6-di(isopropylamino)-4-methoxy-*s*-triazine			Prometon
not shown	2,6-di(isopropylamino)-4-(methylthio)-*s*-triazine			Ametryn

Table III. NMR Spectra of Substituted Pyridines

compd	data
1	0.88 (3 H, t), 1.2–1.4 (6 H), 1.54–1.66 (2 H), 2.59 (2 H, t), 7.19 (1 H, dxd, C(5H), 7.49 (1 H, d, C(4)H), 8.44 superimposed on 8.43 (2 H, C(2)H and C(6)H)
2	similar to 1 but 1.2–1.4 is 8 H
3	similar to 1 but 1.2–1.4 is 10 H
4	0.88 (6 H, d), 1.22 (2 H, mult), 1.50–1.68 (5 H, mult), 2.59 (2 H, t), pyridine signals as 1
5	0.85 (3 H, t), 1.25 (d) superimposed on 1.04–1.35 (6 H together), 1.57 (2 H, mult), 2.70 (1 H, 6 lines), pyridine signals as above
6	0.85 (3 H, t), superimposed on 0.86 (3 H, t), 1.07–1.20 (2 H, mult), 1.25–1.40 (3 H, mult), 1.50–1.70 (2 H, mult), 2.58 (2 H, dxt, J = 1 and 7), 7.20 (1 H, dxd), 7.49 (1 H, br d), 8.41–8.46 (2 H, mult)
7	0.88 (3 H, t), 1.20–1.45 (6 H), 1.65–1.80 (2 H), 2.78 (2 H, t), 7.08 (1 H, dxd, C(5)H), 7.14 (1 H, d, C(3)H), 7.57 (1 H, txd, C(4)H), 8.53 (1 H, d, C(6)H)
8	similar to 7 but 1.20–1.45 is 10 H
9	0.85 (3 H, t), 1.25 (d) superimposed on 1.1–1.35 (6 H together), 1.57 (1 H mult), 1.72 (1 H, mult), 2.86 (1 H, 6 lines), 7.08 (1 H, dxd, C(5)H), 7.12 (1 H, d, C(3)H), 7.58 (1 H, txd, C(4)H), 8.54 (1 H, d, C(6)H)
10	0.88 (6 H, d), 1.22 (2 H, mult), 1.37 (2 H, mult), 1.54 (1 H 7 lines), 1.72 (2 H, mult), 2.78 (2 H, t), pyridine signal as above
11	0.88 (3 H, t), 1.2–1.4 (8 H), 1.54–1.68 (2 H), 2.59 (2 H, t), 7.10 (2 H, d, C(3)H and C(5)H), 8.47 (2 H, d, C(2)H and C(6)H)
12	0.85 (3 H, t), 1.23 (d) superimposed on 1.1–1.35 (6 H together), 1.56 (2 H, mult), 2.66 (1 H, 6 lines), 7.10 (2 H, d, C(3)H), 8.49 (2 H, d, C(2)H)
13	0.89 (3 H, t), 1.25–1.43 (6 H, mult), 1.50–1.62 (2 H, mult), 2.54 (3 H, s), 2.59 (2 H, t), 7.05 (1 H, dxd), 7.39 (1 H, br d), 8.33 (1 H, d + l.r. coupling)
14	0.89 (3 H, t), 1.26–1.42 (6 H, mult), 1.50–1.60 (2 H, mult), 2.30 (3 H, t), 2.59 (2 H, t), 7.04 (1 H, d), 8.28 (1 H, d), 8.31 (1 H, s)
15	0.87 (3 H, t), 1.25–1.4 (6 H), 1.62 (2 H, mult), 2.52 (3 H, s), 2.56 (2 H, t), 7.06 (1 H, d, C(3)H), 7.37 (1 H, dxd, J = 7 and 2), C(4)H), 8.31 (1 H, C(6)H)
16	0.88 (3 H, t), 1.2–1.4 (6 H), 1.60 (2 H, mult), 2.30 (3 H, s), 2.56 (2 H, t), 7.29 (1 H, C(4)H), 8.35 and 8.36 (each 1 H, C(2)H and C(6)H)

superimposed on 5.60 (1 H, two dxd?, C(3)H), 5.78 and 5.80 (1 H together, each dxd, C(4)H, 6.37 and 6.49 (together 1 H, each s, C(6)H). MS: 251 ($M^{\cdot+}$, 4), 236 (30), 208 (10), 192 (32), 164 (100), 148 (3), 134 (20), 120 (38), 107 (33), 106 (90), 93 (38). ^{1}H NMR of **26**: 0.88 (3 H, t), 1.10 and 1.11 (3 H together, each d), 2.0–2.1 (2 H, mult), 4.20 (2 H, 4 lines), 4.58 and 4.72 (1 H together, each q), 5.22 and 5.29 (1 H together, each dxd, C(5)H), 5.60 (overlapping d, C(4)H), 6.51 and 6.61 (1 H together, each d, J = 7 Hz, C(6)H). MS: 251 ($M^{\cdot+}$, 2), 236 (23), 208 (9), 192 (33), 164 (100), 148 (2), 134 (10), 120 (12), 107 (21), 106 (45), 93 (35).

1-(Ethoxycarbonyl)-5-hexyl-2-methyl-1,2-dihydropyridine (**24**, 0.8 g) was treated with chloranil (0.84 g) in toluene (20 mL) at reflux and worked up as before to yield 0.24 g (43%) of 5-hexyl-2-methylpyridine (**15**, 98% pure) with spectral properties as in Table I.

Treatment of 1-(ethoxycarbonyl)-3-hexyl-2-methyl-1,2-dihydropyridine (**26**) with chloranil resulted in complete decomposition. The dehydrogenation was carried out as follows. A tube (70 × 1 cm) was packed with copper chromite (Girdler catalyst G13, powdered) and heated to 250 °C in a pyrolysis oven. 1-(Ethoxycarbonyl)-3-hexyl-2-methyl-1,2-dihydropyridine (**26**, 4 g) was introduced in a current of nitrogen over 4 h, the products being collected in cold traps. Xylene (150 mL) was then passed through the column, and the total collected material was concentrated to yield 120 mg of 3-hexyl-2-methylpyridine (**13**) contaminated with a little 3-hexylpyridine. The 3-hexyl-2-methylpyridine was collected by preparative gas chromatography over Carbowax and identified by spectra as given in Table I.

5-Hexyl-2-methoxy-2-methyl-3,4-dihydro(2*H*)pyran (29). A solution of octanal (100 g, 0.78 mol), formaldehyde (73 g, 36%

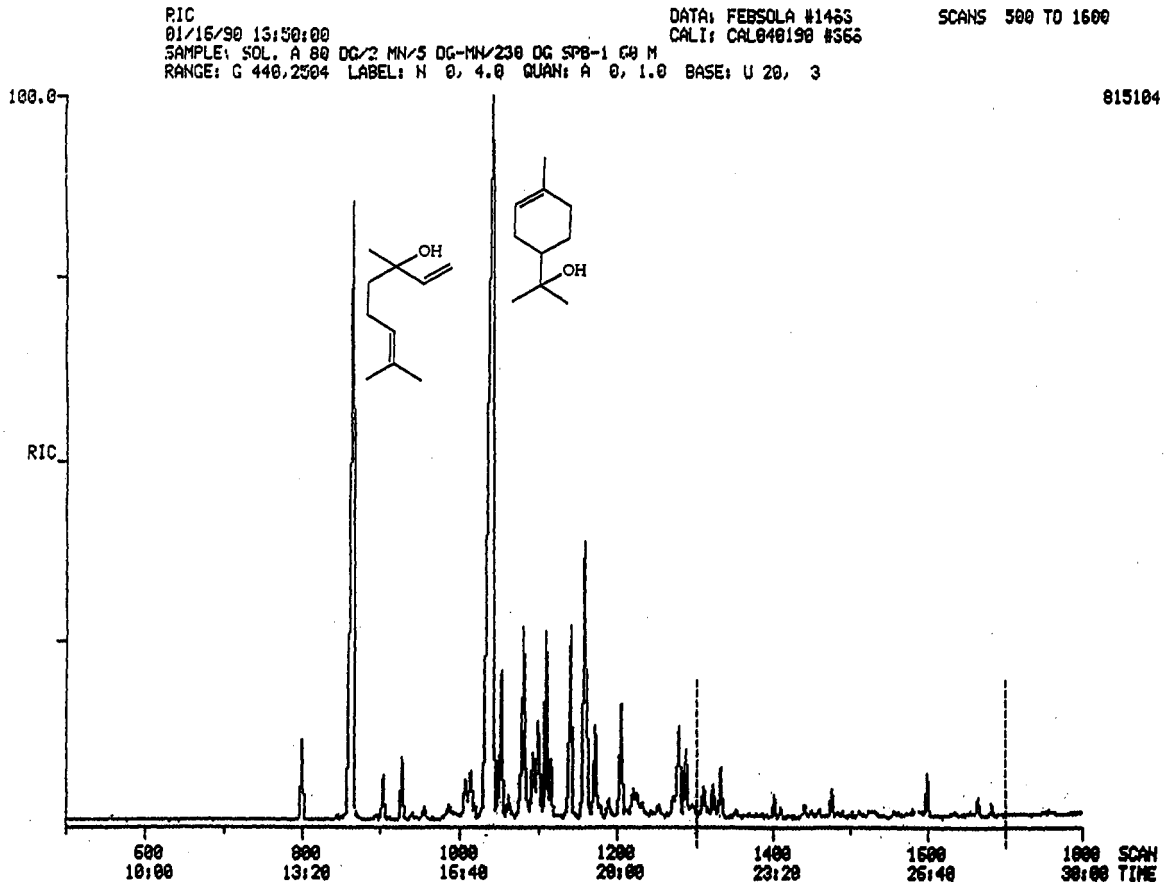


Figure 1. GC of fraction of orange oil extractable by acid. Conditions: SPB-1 column, 60 m, 80 °C, then 5 °C/min to 230 °C. The last part of this chromatogram (scans 1300–1700) is enlarged in Figure 2, and the bases identified are shown.

in water), and diethylamine hydrochloride (260 g, 0.78 mol) was stirred at reflux for 1 h. The mixture was distilled and extracted with ether. The ethereal solution was dried and concentrated to afford 104 g of crude 2-hexylprop-2-enal (**28**), which was used without further purification. 2-Methoxypropene (**27**, 18.5 g, 0.25 mol) was added dropwise over 30 min to a stirred suspension of anhydrous zinc iodide (0.21 g, 0.67 mol) in the crude 2-hexylprop-2-enal (**28**, 42 g, 0.3 mol) at 60 °C under nitrogen. After stirring for a further 6 h, the pale yellow mixture was fractionally distilled to afford the title product (8.2 g, 90% pure by GC, 14%). ^{1}H NMR: 0.88 (3 H, t), 1.38 (3 H, s), 3.25 (3 H, s), 6.03 (1 H, s). MS: 212 ($M^{\cdot+}$, 3), 181 ($M - CH_3O$, 6), 180 (10), 179 (15), 109 (18), 95 (20), 81 (15), 79 (11), 72 (100), 43 (17). This material was used without purification in the next step.

5-Hexyl-2-methylpyridine (15). The crude pyran (**29**, 8.2 g, 38 mmol) was added dropwise over 4 h to a solution of hydroxylamine hydrochloride (2.76 g, 40 mmol), acetic acid (8.6 g, 144 mmol), and water (0.7 g) at 100 °C under nitrogen. After a further 30 min, the cooled reddish-brown mixture was poured into a solution of sodium hydroxide (7.5 g) in ice water (40 g) and the organic phase separated. The aqueous phase was saturated with salt and continuously extracted with ether. Workup of the organic phase yielded 6.01 g of crude product. This was purified by dissolving in sulfuric acid (2 N) and then, after washing with ether, basifying the acid and re-extracting the pyridine. Purification by bulb distillation (120 °C/0.2 mm) yielded 4.18 g (62%) of practically pure material. The spectral properties are described in Tables II and III.

DISCUSSION

Preliminary Qualitative Examination. There was a large amount of non-nitrogenous material present in the fraction containing the bases (apparently water-soluble alcohols and diols). We examined the whole fraction by GC–MS coupling, because injection onto a capillary column fitted with an electron-capture (nitrogen) detector indicated that there were several peaks corresponding to nitrogenous substances, most of them being eluted later than the major peaks of the hydroxylated compounds (Figure 1).

Identification of 3-Alkylpyridines. Most noteworthy were three peaks with retention indices on the 60-m apolar SPB-1 column of 1350, 1455, and 1560 (Figure 2). The mass spectra of these had the most abundant fragment at m/z 93 with the next most abundant fragment at m/z 106, and the apparent molecular ion at, respectively, m/z 163, 177, and 191. These facts would be accommodated by hexyl-, heptyl-, and octylpyridines. We accordingly synthesized 2-heptylpyridine (**7**), 3-heptylpyridine (**2**), and 4-heptylpyridine (**11**). The retention indices of these were 1391 (**7**), 1455 (**2**), and 1460 (**11**), thus eliminating the 2-substituted isomer (**7**) from consideration but leaving a slight doubt between **2** and **11**. The mass spectra of alkyl-substituted pyridines are diagnostic (Budzikiewicz et al., 1969; Porter and Baldas, 1971). Only in the 3-position is the β-cleavage important, leading to the ion a at m/z 92 (Porter and Baldas, 1971). On the other hand, the fission with hydrogen transfer leading to the ion b at m/z 93 is not a good diagnostic test [despite contrary opinion (Porter and Baldas, 1971)], because in all straight-chain-substituted pyridines the most important fragment is at m/z 93. (The mass spectra of all of the pyridines we synthesized are shown in Table I.) We draw attention to the large difference in intensity of the fragment at m/z 120 between the 2- and 4-substituted pyridines, which we feel is most likely due to the stability of the cyclic ion c in the case of the 2-substituted isomers (Scheme I). When the retention times of the synthesized 3-substituted isomers on the SPB-1 column were identical with those of the natural products, we considered their identities as 3-hexyl- (**1**), 3-heptyl- (**2**), and 3-octylpyridine (**3**) to be proved, although

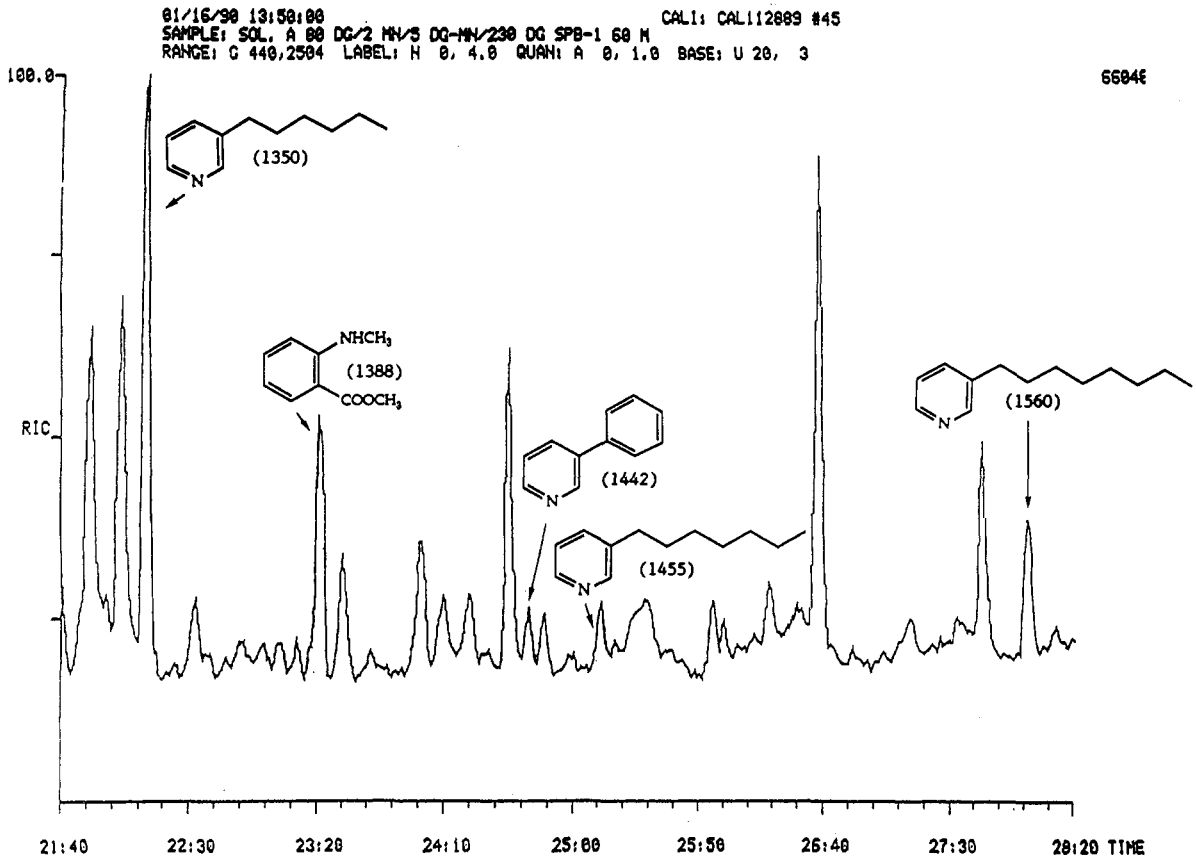
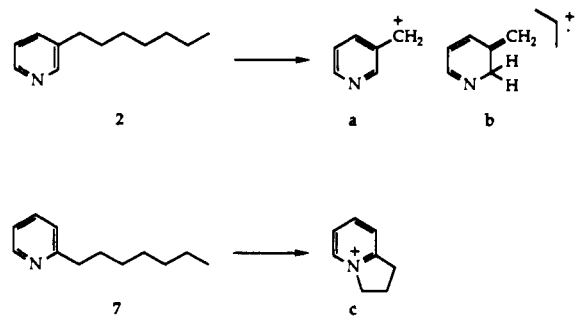


Figure 2. Enlargement of part of Figure 1 (numbers in parentheses are retention indices). Conditions: SPB-1 column, 60 m, 80 °C, then 5 °C/min to 230 °C.

Scheme I



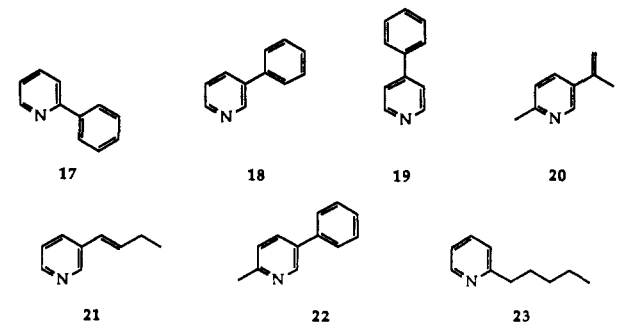


Figure 3.

we did check that the retention times on a polar column (Supelcowax) were also identical.

Identification of 5-Hexyl-2-methylpyridine (15). A further peak with retention index 1412 [just after 3-hexylpyridine (1)] on the apolar column also seemed to be that of a pyridine. With the most important mass spectral fragment at m/z 106 and important fragments at m/z 107 and 120 (but not 93), the most likely attribution would be a methyl-substituted hexylpyridine. The most important fragment could, however, arise either from a branched-chain pyridine (such as 5) or from a hexylpyridine with a methyl group in the ring (such as 15). The branched-chain pyridines (5, 9, 12) could be eliminated because their mass spectra were too different from those of the natural products (see Table I), although the 3-(1-methylhexyl) isomer (5) did have m/z 106 as the most important fragment, unlike the isomers in which the pyridine ring was substituted in the 2- or 4-positions. In view of our finding a series of 3-substituted pyridines, we were naturally tempted to suppose that the unknown hexylmethylpyridine would be a 3-hexylpyridine, especially as the β-cleavage from these isomers (to m/z 106) would be much more important than from other isomers. We synthesized 3-hexyl-2-methyl- (13), 3-hexyl-4-methyl- (14), and 5-hexyl-2-methylpyridine (15); the retention times of 15 and 13 on the apolar column were identical with that of the unknown, but there were distinct differences between the mass spectra (notably the presence of a significant $(M - 1)^+$ fragment in 15 but not in 13 and a relatively more abundant fragment at m/z 107 in 13 than in 15. We finally found that the retention indices of the two pyridines were different on the polar column, the unknown being identical with 5-hexyl-2-methylpyridine (15).

Identification of Phenylpyridines. There were small peaks in the chromatogram (Figure 2) which we ascribed to two of the three phenylpyridines. The retention times of these compounds are fairly close together, but our unknowns had identical retention times with 2-phenylpyridine (17) and 3-phenylpyridine (18), 4-phenylpyridine (19) (Figure 3) having a longer retention time on the apolar column (Rostad and Pereira, 1986). Actually it turned out that all three phenylpyridines were present, but

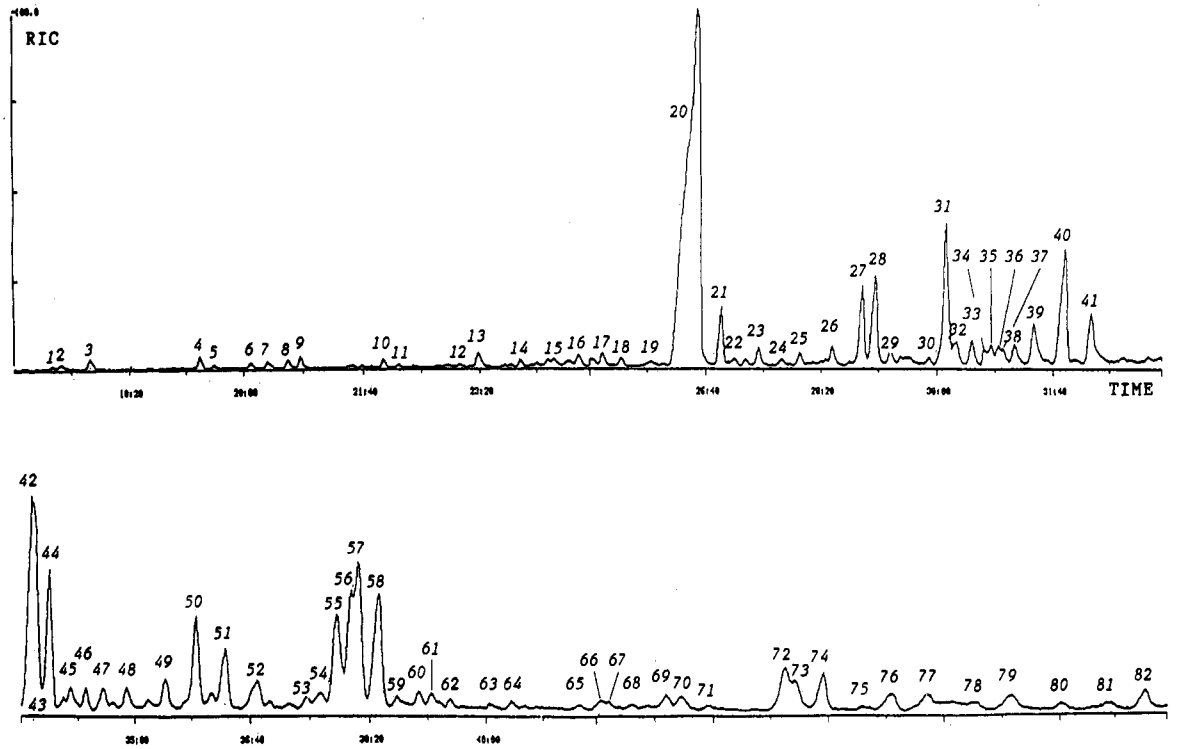


Figure 4. GC of fraction extractable by acid from Florida orange oil. Conditions: Supelcowax column, 60 m, 80 °C, then 5 °C/min to 230 °C.

4-phenylpyridine (**19**) was in too low a concentration to be seen in this extraction.

Quantitative Examination. The main peak in the 8 mg of basic material from Florida oil (see Experimental Procedures) was that of 3-hexylpyridine (**1**) and constituted less than 50% of the total; this corresponds to a maximum of 20 ppb in the original oil. In the extract of the Brazilian oil, 3-hexylpyridine was again the most abundant base, this time constituting much less of the extract, corresponding to a maximum of 15 ppb of the original oil. In the extract of Florida oil, we now identified a number of other bases. The GC on Supelcowax of the extract is shown in Figure 4. Traces of the lower pyridines, 3-ethyl-, 3-propyl-, 3-butyl-, and 3-pentylpyridine, were present but in amounts not exceeding 1 ppb. 2-Methyl-5-(1-methylethenyl)pyridine (**20**) was also identified by MS and retention time [cf. Näf et al. (1980)], as was its isomer, (*E*)-3-(but-1-enyl)pyridine (**21**), with slightly longer retention times on both columns. The latter was previously identified in jonquil absolute (Maurer, 1992).

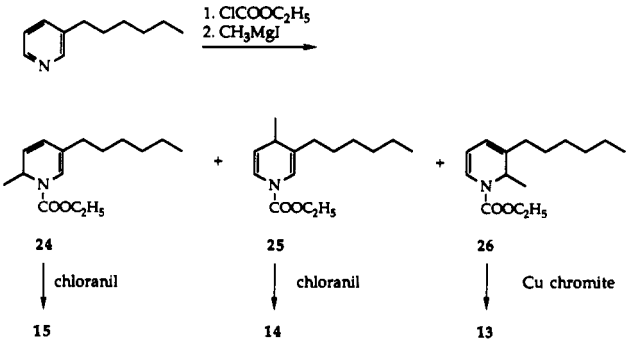
Identification of 3-(4-Methylhexyl)pyridine (**6**). An isomer of 3-hexylpyridine with a shorter retention time on both polar and apolar columns has a mass spectrum identical with that of 3-(4-methylpentyl)pyridine (**4**), a compound we had already synthesized when examining the mass spectra of the pyridines. This compound also had the same retention time as the unknown on both columns, so we consider it to be an authentic constituent of orange oil. Traces of an isomer of 3-heptylpyridine were detected; this isomer had a retention time between those of 5-hexyl-2-methylpyridine (**15**) and 3-heptylpyridine (**2**) on both columns. Because the mass spectrum showed a significant loss of C_2H_5 (at *m/z* 148), we synthesized 3-(4-methylhexyl)pyridine (**6**), which turned out to have a mass spectrum and a retention time identical with those of the unknown.

Occurrence of Pyridines. Table II lists all of the bases definitely identified in the sample in Florida orange oil, most of which are well-known. 2-Methyl-5-phenylpyridine (**22**) was identified by comparison with a commercial sample, and we also noted the presence of traces of other methylated phenylpyridines. The presence of 2-pentylpyridine (**23**, peak 6) seems exceptional, but it is fairly widely occurring in flavors. Because of the similarity of the mass spectra of the *N,N*-dimethyltoluidines, the unknown (peak *11*) was compared with all of them before it was concluded that it was the meta isomer. Having identified ethyl nicotinate (peak 22), we searched for the methyl and propyl esters of nicotinic acid but did not find them. Similarly, when a trace of 5-acetyl-2-methylpyridine (peak *26*) was confirmed, we searched, but in vain, for 3-acetylpyridine. This substance gave a poor MS on the Supelcowax column, where it was eluted with another (unidentified) substance, but it was eluted cleanly on the apolar SPB-1 column. Finally, at long retention times on the apolar column, we observed small peaks with the characteristic mass spectra of Prometon and Ametryn, commercial herbicides, but without authentic material, we could not confirm the identity of the retention times.

Naturally occurring pyridines with longer aliphatic chains are known, for example, 2-heptylpyridine (**7**) from the pedal gland of the bontebok (*Damaliscus dorcas dorcas*) (Burger et al., 1977) and 2-hexylpyridine and homologs from lamb fat (Buttery et al., 1977). 3-Phenylpyridine (**18**) is a well-known natural product occurring in cocoa (Vitzthum et al., 1975; Carlin et al., 1986), the volatiles of alfalfa and red clover (Srinivas, 1988), peppermint and spearmint oils (Sakurai et al., 1983), and French tobacco absolute (Corbier et al., 1988). It is even reported in *Citrus* oils other than that described now (Sakurai et al., 1983). The pyridines with a chain of six or more carbon atoms in the 3-position are, however, new natural products.

5-Acetyl-2-methylpyridine (peak *26*) occurs in artemisia oil (Näf et al., 1980; Näf, personal communication, 1991), although this reference does not say so explicitly. It has also been identified in tobacco smoke (Schmelz and

Scheme II



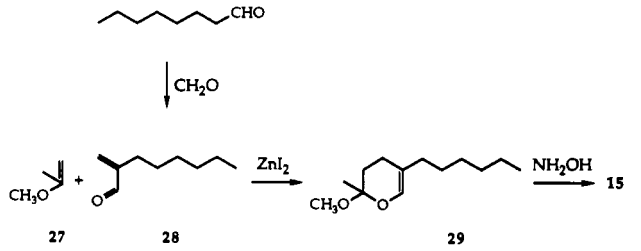
Hoffmann, 1977). It was prepared in 1924 (Benary and Psille, 1924), and we used the sample prepared in this way by Näf et al. (1980).

Synthesis of Pyridines. Monosubstituted pyridines can be prepared from the appropriate picoline (methylpyridine) by the Tchitchibabine method (Tschitschibabin and Seide, 1914; Tchitchibabine, 1936, 1938). This reaction was first applied to 3-picoline by Brown and Murphy (1951); 3-hexyl- (Hardegger and Nikles, 1956), 3-heptyl-, and 3-octylpyridine were made this way (Wibaut and Hoogzand, 1956), 2- and 4-hexylpyridines having been made much earlier (King and Frear, 1944). A careful study of the preparation of 2-, 3-, and 4-alkylpyridines with alkyl from C_2 to C_7 (Kyte et al., 1960) showed that 3-alkylpyridines (in contrast to 2- and 4-alkylpyridines) could only be prepared using ammonia as solvent, and we used this method for all of the straight-chain alkylpyridines. 5-Hexyl-2-methylpyridine (**15**) was synthesized in 1967 from 5-ethynyl-2-methylpyridine (Kost et al., 1967, 1968).

The 3-hexylmethylpyridines (**13–15**) were made initially by quaternization of 3-hexylpyridine with ethyl chloroformate and then addition of methylmagnesium iodide, a reaction first reported by Fraenkel et al. (1970) [see also Lyle and Comins (1976)]. This led to the production of a mixture of the three isomeric intermediates (**24–26**) in the ratio 2:5:10 without catalysis, (Scheme II), this ratio changing to 4:92:4 if cuprous iodide was present, generally in accord with the works of Comins and Abdullah (1982) and Comins and Mantlo (1983). We draw attention, however, to the difference in the proportions of the 2,3- (**26**) and 2,5-substituted (**24**) isomers we obtained in the uncatalyzed reaction. These intermediates were readily separated on a silica gel column, and two (**24**, **25**) were individually converted to the corresponding methylpyridine (**15**, **14**) by chloranil. The dehydrogenation step using chloranil led to complete decomposition in the case of the 3-hexyl-2-methyl isomer (**26**), and using a copper chromite catalyst, only 4% of the pyridine (**13**) was obtained. Although this dehydrogenation gave low yields, sufficient material was available for comparison with the naturally occurring substance.

Having established that the disubstituted hexylpyridine was **15**, we required a more specific synthesis to examine the properties. We based our synthesis on the fact that 1,5-dicarbonyl compounds are converted to pyridines by hydroxylamine (Knoevenagel, 1894), as are 2-alkoxydihydropyrans, their equivalents (Chumakov and Sherstuyuk, 1965). The latter authors had, in fact, prepared 5-hexyl-2-methylpyridine (**15**) from but-3-en-2-one and 1-ethoxyoctene, followed by hydroxylamine (Chumakov and Sherstuyuk, 1965). We chose to start with the enol ether of acetone (**27**) and 2-hexylprop-2-enal (**28**) prepared by a Mannich reaction from octanal. Although the Diels–Alder reaction in the presence of zinc iodide gave poor

Scheme III



yields, the desired dihydropyran (**29**) was obtained cleanly, and treatment with hydroxylamine yielded the pyridine (**15**) uncontaminated with any isomers (Scheme III).

CONCLUSION

Several alkyl and phenylpyridines are therefore present in very small amounts in Valencia and Pera orange oils. The presence of homologs and analogs may suggest that they are not artifacts of extraction.

Various mechanisms have been suggested for the biosynthesis of the pyridine alkaloids which occur in a large variety of plants [see reviews: Gross (1970), Herbert (1974, 1977), and Strunz and Findlay (1985)]. Very schematically alkyl-substituted pyridines could be formed by condensation of aliphatic carbonyl derivatives with ammonia. For example, 2-pentylpyridine (**23**) has been shown to arise from decadienal [known in orange oil (Moshonas and Shaw, 1979)] and ammonia (Buttery et al., 1977; Cramer, 1983). Whether the carbonyl derivatives are already present or if the condensation occurs directly from C_2 or C_3 units with an amino acid are matters for long feeding experiments; different mechanisms occur simultaneously in nature!

Although the odor of 3-butylpyridine was described as early as 1914 as "sweetish, reminiscent of collidine" (Maass and Zablinski, 1914), the organoleptic properties of higher 3-alkylpyridines have not been described. Trained flavorists describe 3-hexylpyridine (**1**) as having a fatty, citrus, orange note, while 5-hexyl-2-methylpyridine (**15**) has fatty, fishy, metallic, and mandarin notes [cf. Thomas and Bassols (1991)]. The flavor threshold concentration of 3-hexylpyridine in water was measured according to the method of Guadagni et al. (1963) by a panel of 13 members as 0.28 ppb, making it one of the most powerful flavoring substances.

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