enzymes (Dauterman, 1971). On the other hand, the low mammalian toxicity of acephate and related *N*-acylphosphoramidothioates has been explained on the basis of their ability to avoid hydrolytic activation to the corresponding phosphoramidothioate, i.e., to the more effective anticholinesterase methamidophos (Kao and Fukuto, 1977; Larson, 1975). If the assumption is made that impurities in malathion and acephate are able to interfere with esterase cleavage of the *O*-acyl or *N*-acyl linkages, respectively, then the impurities would be expected to have a potentiating effect on malathion but an antagonizing effect on acephate. The toxicological data were, in general, consistent with this prediction, indicating that esterases are involved in the potentiation of antagonism of malathion and acephate. Work is currently in progress on the mode of action of the toxicological effects produced by the impurities.

Although there were a number of notable exceptions, on the whole the various impurities identified in technical malathion and acephate were those which might normally be expected in these materials. The formation of impurities, of course, would depend on the industrial method of synthesis. The trimethyl phosphorothioate and phosphorodithioate impurities (A, L, and O) were previously reported to be present in phenthoate and malathion by Pellegrini and Santi (1972) and, therefore, were expected. The presence of L and O, however, is not readily explainable, but these were found in only minor amounts. The relatively large amount of bis(dimethoxyphosphinothioyl) sulfide (B) in technical malathion was somewhat of a surprise since this compound is not attainable through conventional synthetic methods. It may have been formed as a side product in the synthesis of the starting *O,O*-dimethyl phosphorodithioic acid or by the reaction between this acid and malathion. The latter reaction also would explain the formation of diethyl mercaptosuccinate and its coupled products (E, G, H-1). The presence of the mixed carbomethoxy and carboethoxy esters (F) also was surprising. Evidently methanol was present in the reaction mixture for the preparation of malathion, and ester interchange occurred to a small extent. Malathion is prepared on an industrial scale by the addition of *O,O*-dimethyl phosphorodithioic acid to diethyl maleate (Cassady, 1951), and methanol might have been carried forward from the synthesis of the acid.

Acephate is prepared industrially by demethylation and remethylation of *O,O*-dimethyl *N*-acetylphosphoramidothioate (VI) (Magee, 1974). The detection of *O,S*-dimethyl phosphoramidodithioate was, therefore, completely unexpected, and a logical explanation of its presence is not available since this compound contains two sulfur atoms. The presence of *O,O,S*-trimethyl phosphorothioate (VII) also is not obvious. It possibly may be formed by reaction between two molecules of VI (or its precursor *O,O*-dimethyl phosphoroamidothioate) by S-methylation and subsequent attack by the acyl oxygen on the phosphorus atom via a four-center rearrangement.

Storage, particularly at higher temperatures, has a significant effect on the toxicity of technical malathion and acephate to mice, increased toxicity being observed with malathion and decreased toxicity with acephate. Small changes in the composition of the technical materials evidently cause the toxicity differences. Needless to say, malathion should not be stored for prolonged periods under conditions where it is subjected to consistently high temperatures.

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COMMUNICATIONS

2-Ethyl-3-methylbutyric Acid, a New Volatile Fatty Acid Found in Rum

2-Ethyl-3-methylbutyric acid was isolated from rum and identified by coupled GC–MS and IR spectrometry. An NMR spectrum for synthesized 2-ethyl-3-methylbutyric acid is also presented. So far the acid has not been found in any other alcoholic beverage than rum.

Acetic acid forms the major part of the volatile acids in alcoholic beverages, contributing 50–95% in whiskies, about 75% in cognacs and other brandies, and about 80% in rums (Nykänen et al., 1968). These beverages also

contain a number of other volatile fatty acids besides acetic acid. Maarse and ten Noever de Brauw (1966) have shown that Jamaican rum contains several aliphatic, straight-chain fatty acids, including propionic, butyric, and valeric acids. Of the total volatile acids, the proportions of propionic, butyric, and valeric acids are clearly higher in rums than in whiskies and cognacs (Lehtonen and Suomalainen, 1977). Later it was shown that rum also contains branched-chain fatty acids, for example, isobutyric and isovaleric acids (Nykänen et al., 1968; Liebich et al., 1970). Liebich et al. (1970) have also isolated benzoic acid, β-ethoxypropionic acid, and 2- and 3-furancarboxylic acids from Jamaican rum. Among the acids reported in rum by Nykänen et al. (1968) was an unidentified acid. The identification of this component and its isolation from rum is the subject of this report.

EXPERIMENTAL SECTION

Isolation of the Acid Fraction. The volatile fatty acids were isolated from Martinique rum using the method developed by Nykänen et al. (1968).

Isolation and Identification of 2-Ethyl-3-methylbutyric Acid. The acid was isolated from the acid fraction of rum on a Hewlett Packard 7620A gas chromatograph fitted with a TC-detector using a 2 m × $^1/_4$ in. stainless steel column of 20% butanediol succinate and 2.5% phosphoric acid on HMDS-treated, acid-washed Chromosorb W, 60–80 mesh. For the mass spectrometry, the 2-ethyl-3-methylbutyric acid was also fractionated as its methyl ester using a 3 m × $^1/_4$ in. stainless steel column of 10% FFAP on acid-washed Chromosorb W, 60–80 mesh. The acids were methylated with diazomethane.

The IR spectrum of the isolated acid was run as a thin film between KBr discs on a Perkin-Elmer 521 infrared spectrophotometer. The mass spectrum of the methyl ester was run at the Finnish State Technical Research Center on their JEOL JMS-D 100 combined GC–MS.

Synthesis of Reference Compound. Because it was not available commercially, 2-ethyl-3-methylbutyric acid was synthesized from methoxyacetonitrile using the method of Barnes and Budde (1946). By two successive Grignard reactions 1-methoxy-2-ethyl-3-methyl-2-butanol was prepared from methoxyacetonitrile via 1-methoxy-2-butanone. Upon heating with anhydrous oxalic acid, this alcohol formed 2-ethyl-3-methylbutanal, which was oxidized to the corresponding acid by alkaline silver oxide. A Fourier transform NMR spectrum of the synthesized acid in $CDCl_3$ solution using Me_4Si as internal standard was obtained at the Organic Chemistry Department of Helsinki University using their JEOL JMM PFT 100 NMR spectrometer.

RESULTS AND DISCUSSION

Figure 1 presents gas chromatograms of the lower fatty acid composition of Martinique rum, Jamaican rum, Scotch whisky, and cognac. It can be clearly seen that 2-ethyl-3-methylbutyric acid appears only in the rum chromatograms, and it has not yet been identified in any other alcoholic beverage. The content of 2-ethyl-3-methylbutyric acid in rum is comparable with that of hexanoic acid. The acid has also been identified in other types of rum besides the heavy Martinique and Jamaican rums, for example, in Bacardi rum.

Infrared spectra of the synthesized 2-ethyl-3-methylbutyric acid and of the acid isolated from rum are shown in Figure 2. The spectra are identical. Other typical vibrations besides those of the OH group (3100 cm^{-1}) and the carbonyl group (1700 cm^{-1}) appear in the spectra. The

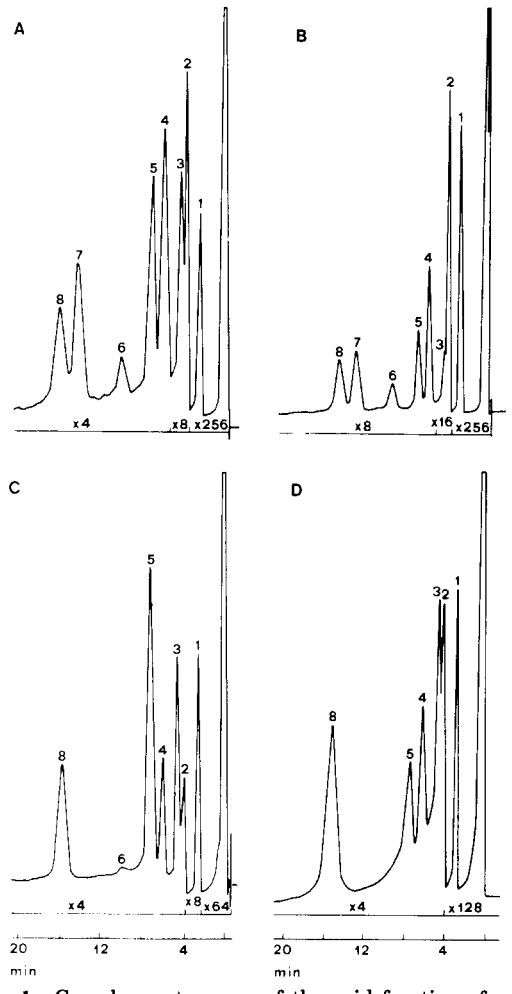

Figure 1. Gas chromatograms of the acid fractions from (A) Martinique rum, (B) Jamaican rum, (C) Scotch whisky, and (D) authentic French cognac: (1) acetic acid, (2) propionic acid, (3) isobutyric acid, (4) butyric acid, (5) isovaleric acid, (6) valeric acid, (7) 2-ethyl-3-methylbutyric acid, (8) hexanoic acid; column, 20% butanediol succinate and 2.5% H_3PO_4; oven temperature, isothermal at 144 °C.

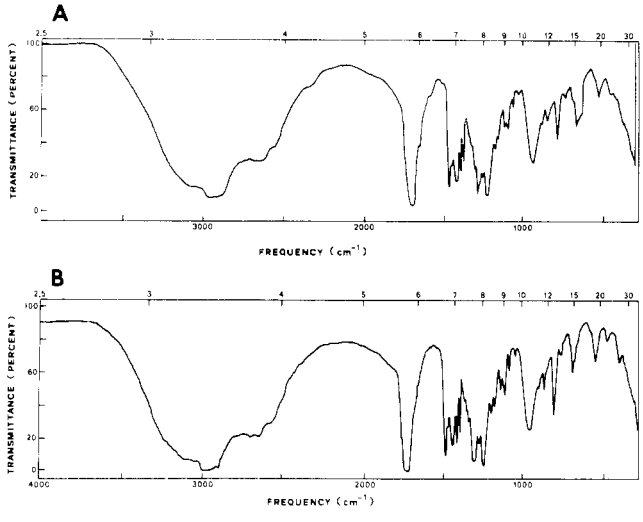

Figure 2. Infrared spectra of (A) the acid isolated from rum, and (B) 2-ethyl-3-methylbutyric acid. The spectra were run as thin films between KBr discs.

double peak at wave numbers 1385 and 1368 cm^{-1} of asymmetric CH_3 bending is characteristic for the presence of the *gem*-dimethyl group, as are the skeletal vibrations

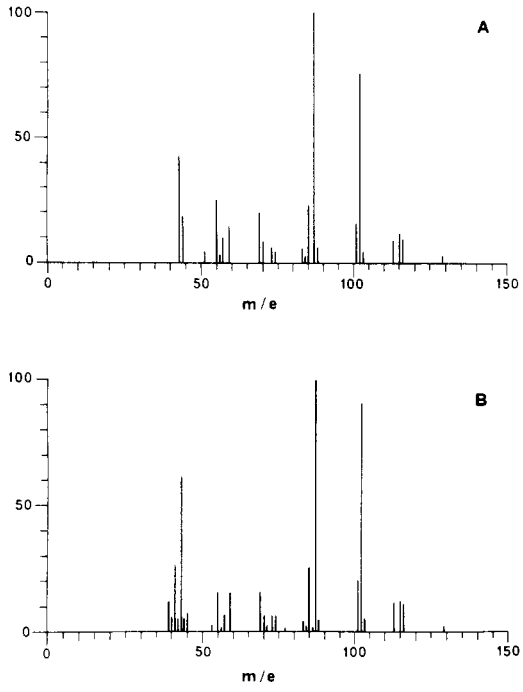

Figure 3. Mass spectra of (A) the methyl ester of the acid isolated from rum, and (B) the methyl ester of 2-ethyl-3-methylbutyric acid.

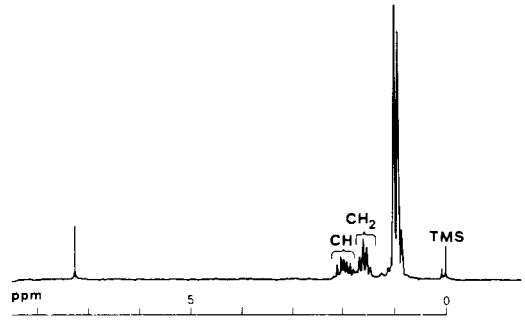

Figure 4. NMR spectrum of 2-ethyl-3-methylbutyric acid.

at wave numbers 1170 and 1150 cm^{-1}. The presence of an ethyl substituent is shown by the rocking of the ethyl CH_2 at wave number 780 cm^{-1} (Nakanishi, 1962).

The identity of the synthesized 2-ethyl-3-methylbutyric acid and the acid isolated from rum was confirmed by the mass spectra of the methyl esters shown in Figure 3. The strong peak of the $(M - 42)^+$ ion at mass number m/e 102 derives from the neutral C_3H_6 fragment being split off from the molecule in a McLafferty rearrangement (Hamming and Foster, 1972). The base peak at mass number m/e 87 is obtained by the subsequent loss of a CH_3 group from the $(M - 42)^+$ ion. The peak at mass number m/e 43 is assigned to the C_3H_7 ion fragment. The relatively weak peak of the $(M - 31)^+$ ion at mass number m/e 113 is a result of the fragmentation of a methoxyl group, and the weak peak of the $(M - 15)^+$ ion at mass number m/e 129 is from the fragmentation of a neutral CH_3 group.

Figure 4 shows the NMR spectrum of the synthesized 2-ethyl-3-methylbutyric acid.

The ultimate source of the acid is not yet known, but its appearance in rums and not in other alcoholic beverages may provide a lead for subsequent investigations.

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Matti J. Lehtonen*
Brita K. Gref
Erkki V. Puputti
Heikki Suomalainen

Research Laboratories of the State Alcohol Monopoly (Alko), SF-00101 Helsinki 10, Finland

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Analysis of Mixtures of Solanidine and Demissidine Glycoalkaloids Containing Identical Carbohydrate Units

Mixtures of solanidine-based glycoalkaloids and demissidine-based glycoalkaloids were hydrolyzed under conditions that quantitatively yield solanthrene from the former and demissidine from the latter. The method has been applied to the analysis of potato tuber samples that contain glycoalkaloids differing only in the structure of the aglycone, i.e., solanidine vs. demissidine.

Plants of the *Solanum* genus contain potentially toxic compounds which are carbohydrate derivatives of 3-hydroxysteroidal alkaloids. These compounds are commonly referred to as glycoalkaloids. Their presence in edible plants such as potato, tomato, and eggplant has been of concern because of their toxicity. Although they are usually found at levels that are not toxic, isolated instances of illness and even death (Hansen, 1925) have been ascribed to potato glycoalkaloids.

Much research has been devoted to the identification (Schrieber, 1968) and quantitation (Bretzloff, 1971; Fitzpatrick and Osman, 1974) of glycoalkaloids, particularly in edible plants. Most of the recent qualitative analysis has been done by thin-layer chromatography