from this sample had less than 10% of the ionophoric activity of an equivalent amount of salinomycin. This degree of ionophoric activity represents the maximum observed, while that obtained with the remaining samples was nearly negligible. The low ^{86}Rb binding levels in the treated samples are likely due to the very low levels of unchanged salinomycin in these samples and the high degree of polarity of the salinomycin metabolites in pig liver (data not presented) resulting in a loss of ionophoric activity. The levels of ^{86}Rb binding obtained with the two control samples were between those obtained with the 0 and 63 ppb [^{14}C]salinomycin standards.

CONCLUSIONS

Even though the residue levels in liver are at the tolerance limit of 1800 ppb, their toxicological consequences would be minimal as reflected by their lack of ionophoric activity, since only 2 of the 12 liver samples from treated pigs had ionophoric activity greater than twice that observed with the control samples. Of these two liver samples, the extractable residue had less than 10% of the ionophoric activity of an equivalent amount of salinomycin.

LITERATURE CITED

Dimenna, G. P.; Lyon, F. S.; Thompson, F. M.; Creegan, J. A.; Wright, G. J. "Effect of Antibiotic Combination, Dosing Period, Dose Vehicle, and Assay Method on Salinomycin Residue Levels and Their Ionophoricity in Chicken Tissues". *J. Agric. Food Chem.* **1989**, *37*, 668-676.

Lindemann, M. D.; Stahly, T. S.; Cromwell, G. L.; Easter, R. A.; Kerr, B. J.; Lucas, D. M. The Efficacy of Salinomycin as a Growth Promotant for Swine From 9 to 97 kg. *J. Anim. Sci.* **1985**, *61*, 782-788.

McClure, W. N.; Fontenot, J. P.; Webb, K. E., Jr.; Lucas, D. M. Feedlot performance of cattle fed different salinomycin levels. *J. Anim. Sci.* **1980**, *51*, 380.

National Research Council. *Nutrient Requirements of Swine*, 8th ed. (revised); National Academy of Sciences: Washington, DC, 1979.

Reed, P. W. Biochemical and Biological Effects of Carboxylic Acid Ionophores. In *Polyether Antibiotics. Naturally Occurring Acid Ionophores. Volume I: Biology*; Westley, J. W., Ed.; Marcel Dekker: New York, 1982.

Received for review September 15, 1989. Accepted January 17, 1990.

Registry No. Salinomycin, 53003-10-4.

Structure Elucidation of the Product Prepared from the Reaction of 3-Methyl-5,6-dihydro-2(1*H*)-pyrazinone and Ketones or Aldehydes

Satoru Mihara* and Hideki Masuda

Ogawa & Company, Ltd., 6-32-9 Akabanenishi, Kita-ku, Tokyo 115, Japan

The condensation products prepared from reactions of 3-methyl-5,6-dihydro-2(1*H*)-pyrazinone (1) and ketones or aldehydes had previously been assigned the structures 5-alkyl-3-methyl-2(1*H*)-pyrazinones **3**. Current work revealed that the formulas should be revised to 6-alkyl-3-methyl-2(1*H*)-pyrazinones **2**.

Some alkoxy- and (alkylthio)pyrazines derived from 2(1*H*)-pyrazinones (Masuda et al., 1981; Masuda and Mihara, 1986) showed high potential use as flavor ingredients (Fors, 1983). Although 5-alkyl-3-methyl-2(1*H*)-pyrazinones **3** prepared from the reaction of 3-methyl-5,6-dihydro-2(1*H*)-pyrazinone (1) and ketones or aldehydes have been reported (Masuda et al., 1981), their chemical structures were not fully examined. Determination of the structure of these compounds by ^1H NMR spectra is not necessarily straightforward (MacDonald et al., 1976). We now report that NOE difference experiments and X-ray crystallography demonstrate that these compounds are actually 6-alkyl-3-methyl-2(1*H*)-pyrazinones **2** (see Scheme I).

EXPERIMENTAL SECTION

Instrumentation. The IR, ^1H NMR, and GC/MS were recorded on a Hitachi 260-10, a Bruker AM-400, and a Hitachi M-80B spectrometer, respectively. GC analyses were carried out on a Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector and a fused-silica capillary column coated with Carbowax 20M or OV-101.

Synthesis of 3-Methyl-2(1*H*)-pyrazinone (2'). This compound was prepared by the method described by Masuda and Mihara (1986).

Derivation of 2-Isopropyl-5-methylpyrazine (5a) from 6-Isopropyl-3-methyl-2(1*H*)-pyrazinone (2a) (See Scheme II). Compound **2a**, prepared as described by Masuda et al. (1981) (2 g, 0.013 mol), and phosphorus oxychloride (20 g, 0.13 mol) were refluxed for 2 h. The reaction mixture was then gradually added to ice-cooled water (100 mL). The reaction mixture was adjusted to pH 8 with 50% NaOH. After the insoluble material was filtered off, the filtrate was concentrated under vacuum. The oily residue (2.3 g) was further distilled in vacuo to give 3-chloro-5-isopropyl-2-methylpyrazine (**4a**; 1.7 g, 0.01 mol); bp 68 °C (2 mm) (Karmas and Spoerri, 1952).

A dioxane solution (7 mL) of **4a** (1.7 g, 10 mmol) was hydrogenated in the presence of Pd/C (10%, 0.2 g) and sodium meth-

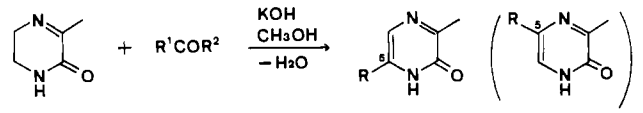
0021-8561/90/1438-1032$02.50/0

Table I. Spectral Data of 2-Alkyl-5-methylpyrazines 5

no.	IR (neat): ν, cm^{-1}	NMR ($CDCl_3$): δ	MS: m/z, %	ref
5a	2950, 2920, 2860, 1480, 1450, 1380, 1290, 1250, 1160, 1140, 1030, 890	1.32 (6 H, d, J = 6.9 Hz, $CH(CH_3)_2$), 2.53 (3 H, br s, CH_3), 3.08 (1 H, septet, J = 6.9 Hz, $CH(CH_3)_2$), 8.36 (1 H, d, J = 1.5 Hz, ring H), 8.37 (1 H, m, ring H)	136 (M^+, 30), 135 (19), 122 (8), 121 (100), 108 (42), 94 (8), 67 (6), 59 (9)	*a*, *b*
5b	2960, 2930, 2870, 1480, 1450, 1380, 1310, 1250, 1160, 1140, 1030, 890, 800	0.84 (3 H, t, J = 7.5 Hz, CH_2CH_3), 1.29 (3 H, d, J = 6.9 Hz, $CHCH_3$), 1.65 (1 H, ddq, J = 13.4, 6.4, 7.5 Hz, $CHHCH_3$), 1.76 (1 H, ddq, J = 13.4, 7.5, 7.5 Hz $CHHCH_3$), 2.53 (3 H, br s, CH_3), 2.80 (1 H, m, $CHCH_3$), 8.31 (1 H, d, J = 1.4 Hz, ring H), 8.39 (1 H, m, ring H)	150 (M^+, 14), 135 (40), 123 (8), 122 (100), 121 (51), 108 (26), 107 (5), 94 (6), 93 (5), 66 (4)	*a*
5c	2950, 2930, 2870, 1490, 1470, 1380, 1350, 1330, 1250, 1160, 1040, 900, 750	0.94 (6 H, d, J = 6.7 Hz, $CH(CH_3)_2$), 2.08 (1 H, m, $CH(CH_3)_2$), 2.54 (3 H, br s, CH_3), 2.64 (2 H, d, J = 7.2 Hz, $ArCH_2$), 8.30 (1 H, d, J = 1.5 Hz, ring H), 8.38 (1 H, m, ring H)	150 (M^+, 9), 135 (14), 109 (8), 108 (100), 107 (8), 80 (5)	*a*, *c*
5d	2950, 2930, 2870, 1480, 1450, 1380, 1350, 1330, 1160, 1030, 750	0.87 (3 H, d, J = 6.7 Hz, $CHCH_3$), 0.92 (3 H, t, J = 7.3 Hz, CH_2CH_3), 1.24 (1 H, ddq, J = 13.1, 7.3, 7.3 Hz, $CHHCH_3$), 1.41 (1 H, ddq, J = 13.1, 5.7, 7.3 Hz, $CHHCH_3$), 1.87 (1 H, m, $CHCH_3$), 2.54 (3 H, br s, CH_3), 2.54 (1 H, dd, J = 13.5, 8.3 Hz, $ArCHH$), 2.79 (1 H, dd, J = 13.5, 6.2 Hz, $ArCHH$), 8.30 (1 H, d, J = 1.4 Hz, ring H), 8.39 (1 H, m, ring H)	164 (M^+, 2), 149 (4), 135 (6), 121 (3), 108 (100), 80 (3), 57 (2)	

[a] Bramwell et al. (1971). [b] Bondarovich et al. (1967). [c] Vitzthum et al. (1975).

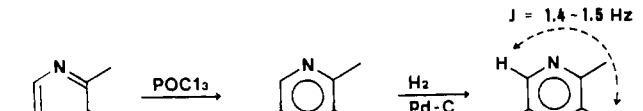
Scheme I



1 2 3

a : R = $CH(CH_3)_2$
b : R = $CH(CH_3)C_2H_5$
c : R = $CH_2CH(CH_3)_2$
d : R = $CH_2CH(CH_3)C_2H_5$

Scheme II



2 4 5

a : R = $CH(CH_3)_2$
b : R = $CH(CH_3)C_2H_5$
c : R = $CH_2CH(CH_3)_2$
d : R = $CH_2CH(CH_3)C_2H_5$

oxide (5 mmol) (Bramwell and Wells, 1972). The calculated amount of H_2 was absorbed in 4 h. After the precipitate was removed by filtration, the filtrate was concentrated. Subsequent preparative TLC (eluting with 1:6 ethyl acetate–methylene chloride; R_f 0.28) gave **5a** (1.1 g, 80%) as a colorless oil. The spectral data of this compound were the same as that of **5a** obtained by Bramwell et al. (1971) as shown in Table I.

2-Methyl-5-(1′-methylpropyl)-, 2-Isobutyl-5-methyl-, and 2-Methyl-5-(2′-methylbutyl)pyrazine (5b–d). Pyrazinones **2b–d** were synthesized by the same reaction used to prepare **2a** (Masuda et al., 1981). The reaction mixture was extracted with ethyl acetate. The solvent was removed from the extract, and the residual solid, namely the crude product, was recrystallized from ethyl acetate. The yields of the crude and pure products (**2a–d**) are shown in Table II. **5b–d** were prepared from the corresponding pyrazinones (**2b–d**) by the same method to prepare **5a** (see Scheme II). The spectral data of **5a–d** were given in Table I.

Table II. Comparison of the Yields for the Crude and Pure 6-Alkyl-3-methyl-2(1H)-pyrazinones 2

	6-alkyl-3-methyl-2(1*H*)-pyrazinone	yield, % crude	yield, % pure[a]
2a	6-isopropyl-3-methyl-2(1*H*)-pyrazinone	25	22
2b	3-methyl-6-(1′-methylpropyl)-2(1*H*)-pyrazinone	19	17
2c	6-isobutyl-3-methyl-2(1*H*)-pyrazinone	69	65
2d	3-methyl-6-(2′-methylbutyl)-2(1*H*)-pyrazinone	55	52

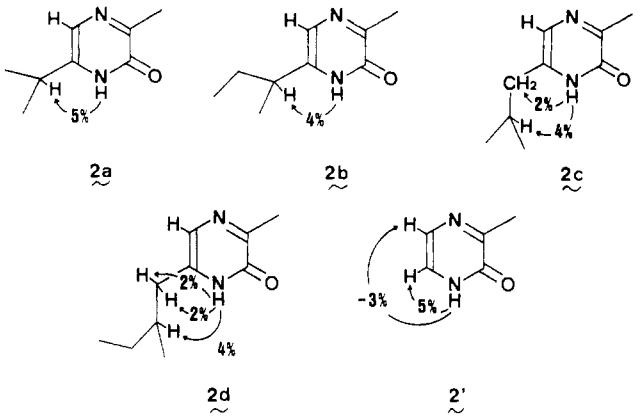
[a] Masuda et al. (1981).

Crystallographic Data and X-ray Structure Analysis of 2a. (See the supplementary material.) Crystals of **2a** are irregular plates from ethyl acetate, triclinic space group $P\bar{1}$; a = 9.568 (1) Å, b = 10.298 (1) Å, c = 4.364 (1) Å; α = 98.75 (1)°, β = 87.80 (1)°, γ = 98.22 (1)°; V = 420.6 (1) $Å^3$; Z = 2. Three-dimensional intensity data were collected on a Rigaku AFC-5FOS automated four-circle diffractometer [Mo Kα radiation, λ = 0.710 679 Å, graphite monochromator, θ–2θ scan method, $2\theta_{max}$ = 60°, scan range $\Delta(2\theta)$/deg = 1.4 + 0.7 tan θ, scan speed in 2θ = 12°/min]. The total number of reflections measured was 2747, of which 1071 were used in the structure refinement. The solution and refinement of the structure were carried out on a FACOM S3500 superminicomputer at the Material Analysis Center, ISIR, Osaka University. The structure was reduced by direct methods (MULTAN 84), refined by block-diagonal least-squares methods to a final R factor of 0.060.

RESULTS AND DISCUSSION

Only one peak appeared in the gas chromatogram of the crude condensation product of 1 and ketones or aldehydes. The yields of the crude and pure products are very close each other, as shown in Table II. It may be concluded that the reaction product is mostly one kind of product.

High-resolution MS demonstrated that the condensation product **2a** has a molecular formula of $C_8H_{12}N_2O$.



2a 2b 2c 2d 2'

Figure 1. Difference NOEs between the NH proton and other protons in **2a–d** and 2'.

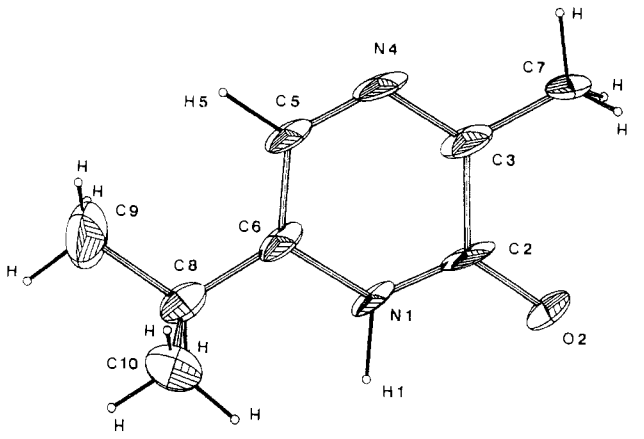


Figure 2. ORTEP view of **2a**. The non-hydrogen atoms were drawn at the 50% probability level and the hydrogen atoms at an arbitrary diameter.

The IR spectrum showed the absorption bands at 1650 and 1530 cm^{-1} (CONHR). The ^1H NMR spectrum ($CDCl_3$) showed the presence of a geminal dimethyl proton at δ 1.27 (6 H, d, J = 7.0 Hz), a methyl proton attached to an olefinic carbon at δ 2.35 (3 H, s), a methine proton at δ 2.78 (1 H, septet, J = 7.0 Hz) and a proton adjacent to nitrogen at δ 7.10 (1 H, s), and a hydrogen-bonded CONH proton at δ 12.55 (1 H, br s). It was concluded from these spectral data that the structure of the condensation product was **2a** or **3a** (see Scheme I). In order to differentiate between these two possibilities, NOE difference experiments were carried out. Irradiation of the H-1 broad singlet at δ 12.55 gave a 5% increase of the septet at δ 2.78 assigned to the $(CH_3)_2HCC$-6 proton. This NOE interactions established that structure of the condensation product is best assigned as **2a**. The observed NOE values for **2a–d** and 3-methyl-2(1*H*)-pyrazinone (**2'**) are given in Figure 1.

In order to confirm the structure of **2a**, we have prepared isopropylmethylpyrazine **5a** from **2a** as shown in Scheme II. The ortho, para, and meta coupling constants for the ring protons in 2,3-, 2,5-, and 2,6-dialkyl-substituted pyrazines have shown values in the ranges 2.4–2.9, 1.3–1.6, and −0.5 to 0 Hz, respectively (Bramwell et al., 1971). The assignment of **5a** as the 2,5-isomer on the basis of the coupling constant was verified by unequivocal methods of syntheses. Similarly, **5b–d** were synthesized from the corresponding **2b–d** (see Scheme II), and their spectral data were given in Table I.

The molecular structure of **2a** was determined unambiguously by X-ray crystallography and in agreement with the structure proposed by the above NMR arguments (Figure 2). Consequently, it is shown that 6-alkyl-3-methyl-2(1*H*)-pyrazinones **2** can be synthesized by the reaction of 3-methyl-5,6-dihydro-2(1*H*)-pyrazinone (**1**) and ketones or aldehydes.

ACKNOWLEDGMENT

We thank T. Tanaka of the Material Analysis Center, ISIR, Osaka University, for the X-ray analysis.

Supplementary Material Available: Complete data for X-ray crystal structure including tables of atomic positional and equivalent isotropic thermal parameters, anisotropic thermal parameters, atomic distances, and bond angles and a figure showing the numbering of atoms for **2a** (7 pages); listings of observed and calculated structure factors (8 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Bondarovich, H. A.; Friedel, P.; Krampl, V.; Renner, J. A.; Shephard, F. W.; Gianturco, M. A. Volatile Constituents of Coffee. Pyrazines and Other Compounds. *J. Agric. Food Chem.* **1967**, *15*, 1093.

Bramwell, A. F.; Wells, R. D. The Nuclear Magnetic Resonance Spectra of Pyrazines. *Tetrahedron* **1972**, *28*, 4155.

Bramwell, A. F.; Payne, L. S.; Riezebos, G.; Ward, P.; Wells, R. D. The Nuclear Alkylation of Pyrazines by Ketones and Aldehydes. *J. Chem. Soc. C* **1971**, 1627.

Fors, S. *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society; Washington, DC, 1983; p 185.

Karmas, G.; Spoerri, P. E. The Preparation of Hydroxypyrazines and Derived Chloropyrazines. *J. Am. Chem. Soc.* **1952**, *74*, 1580.

MacDonald, J. C.; Bishop, G. G.; Mazurek, M. ^{13}C and Proton NMR Spectra of 2(1*H*)-Pyrazinones. *Tetrahedron* **1976**, *32*, 655.

Masuda, H.; Mihara, S. Synthesis of Alkoxy-, (Alkylthio)-, Phenoxy-, and (Phenylthio)pyrazines and Their Olfactive Properties. *J. Agric. Food Chem.* **1986**, *34*, 377.

Masuda, H.; Yoshida, M.; Shibamoto, T. Synthesis of New Pyrazines for Flavor Use. *J. Agric. Food Chem.* **1981**, *29*, 944.

Vitzthum, O. G.; Werkhoff, P.; Hubert, P. Volatile Components of Roasted Cocoa: Basic Fraction. *J. Food Sci.* **1975**, *40*, 911.

Received for review April 26, 1989. Accepted December 28, 1989.

Registry No. 2', 19838-07-4; **2a**, 125781-24-0; **2b**, 125781-25-1; **2c**, 125781-27-3; **2d**, 125781-28-4; **3a**, 78246-12-5; **4a**, 116513-21-4; **4b**, 125781-26-2; **4c**, 115978-65-9; **4d**, 125781-29-5; **5a**, 13925-05-8; **5b**, 29461-07-2; **5c**, 29461-05-0; **5d**, 125781-30-8.