

Hydroxy Nitriles in Blackcurrant Buds Absolute (*Ribes nigrum* L.)

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Four hydroxy nitriles have been identified in blackcurrant buds absolute (*Ribes nigrum* L.). These were the 3-hydroxy-2-methylbutyronitrile (diastereomer) and (*E*)- and (*Z*)-2-(hydroxymethyl)-2-butenonitrile. The structures of these compounds were determined by spectroscopic methods.

Blackcurrant (*Ribes nigrum* L.) is an important raw material for the food industry in Central and Northern Europe. The blackcurrant buds absolute, which have a very characteristic and powerful odor, serve as a flavor enhancer and, in recent years, have been used as a major ingredient in some luxury fragrances. The volatile compounds of the blackcurrant buds have been previously investigated (Kerslake and Menary, 1985; Derbesy et al., 1982; Latrasse and Lantin, 1977; Williams, 1972). Recently, 2-mercapto-4-methoxy-2-methylbutane was identified in the essential oil of blackcurrant buds as the compound responsible for the "catty" note of the buds (Rigaud et al., 1986). In this paper, we describe the isolation and identification of four hydroxy nitriles that have been newly identified in nature.

EXPERIMENTAL SECTION

Infrared spectra (IR) were obtained with use of a Hitachi Model 260-10 grating infrared spectrometer. NMR spectra were obtained in CDCl₃ on a Bruker AM-400 spectrometer operating at 400.139 MHz (¹H) or 100.679 MHz (¹³C) for samples in a 5-mm tube (¹H/¹³C). Chemical shifts were measured relative to Me₄Si as an internal standard. A Hitachi Model 663-30 gas chromatograph equipped with a dual FID/FTD and a 50-m length × 0.22-mm i.d. fused silica capillary column coated with Carbowax 20M was used for routine analysis. The oven temperature was programmed from 80 to 200 °C at 2 °C/min. The nitrogen carrier flow rate was 0.67 mL/min. The injector and detector temperatures were 250 °C. The injector split ratio was 1:50. A Hewlett-Packard Model 3385A integrator was used to determine the peak areas. Mass spectral analysis [electron impact (EI), chemical ionization (CI), high resolution (HR)] were performed by the Hitachi Model M-80 combination mass spectrometer-gas chromatograph (Hewlett-Packard Model 5710A) equipped with a Hitachi Model M-6010 and M-003 data system. The gas chromatographic and oven conditions were similar to those described for the Hitachi instrument. The conditions were as follows: electron energy, 70 eV; ion source temperature, 200 °C; electron multiplier voltage, 3100 V. For chemical ionization, NH₃ was used as the reagent gas. Preparative gas chromatography was performed on a Hitachi 5201 gas chromatograph equipped with a thermal conductivity detector and a glass column (2-m length × 4-mm i.d.) packed with 5% Carbowax 20M on Chromosorb WAW DMCS (60/80). The oven temperature was programmed from 170 to 200 °C at 5 °C/min. The preparative HPLC was performed on an instrument equipped with a refractive index detector and a GPC column using chloroform as an eluant.

Fractionation of the Blackcurrant Buds Absolute. A 154-g portion of blackcurrant buds absolute (*R. nigrum*

L.) supplied by Cammilli, Albert & Laloue, Grasse, France, was mixed with 1 kg of water and the resultant mixture simultaneously steam distilled and extracted with 300 mL of methylene chloride (CH₂Cl₂ layer 1) for 12 h on a modified Likens and Nickerson apparatus (Schultz et al., 1977). The aqueous sample pot contents was then extracted with 300 mL of methylene chloride (CH₂Cl₂ layer 2) on a liquid-liquid continuous extractor for 8 h. After drying (Na₂SO₄), the extracts (CH₂Cl₂ layers 1 and 2) were concentrated on a rotary evaporator.

Isolation of Hydroxy Nitriles. The concentrate of CH₂Cl₂ layer 2 was distilled under reduced pressure [90 °C (5-10 mmHg)]. The fractions containing hydroxy nitriles were finally purified by preparative GLC to give a mixture of peaks A and B (7 mg, A/B = ca. 1/2, yield 0.0045%), C (10 mg, yield 0.0065%), and D (5 mg, yield 0.0032%). The spectral data of the hydroxy nitriles are summarized below.

Compounds A and B (peaks A and B; ca. 1/2): IR ν_{\max} (neat, cm⁻¹) 3425, 2985, 2925, 2260, 1735, 1640, 1460, 1385, 1120, 1030, 1000, 935, 870; MS (A) m/z 84 (M⁺ - 15, 3%), 56 (18), 55 (53), 54 (20), 46 (2), 45 (100); MS (B) m/z 84 (M⁺ - 15, 2%), 56 (16), 55 (47), 54 (18), 46 (2), 45 (100); CIMS (A; reagent gas NH₃) m/z 117 (M + NH₄)⁺; CIMS (B; reagent gas NH₃) m/z 117 (M + NH₄)⁺; ¹H NMR (A; CDCl₃; Me₄Si) δ 1.36 (3 H, d, J = 6.3 Hz, CH₃CHOH), 1.37 (3 H, d, J = 7.2 Hz, CH₃CHCN), 1.74 (1 H, br s, OH), 2.66 (1 H, qd, J = 7.2 and 4.7 Hz, CHCN), 3.87 (1 H, qd, J = 6.3 and 4.7 Hz, CHOH); ¹H NMR (B; CDCl₃; Me₄Si) δ 1.32 (3 H, d, J = 7.2 Hz, CH₃CHCN), 1.37 (3 H, d, J = 6.3 Hz, CH₃CHOH), 1.74 (1 H, br s, OH), 2.76 (1 H, qd, J = 7.2 and 5.6 Hz, CHCN), 3.91 (1 H, qd, J = 6.3 and 5.6 Hz, CHOH); ¹³C NMR (A; CDCl₃; Me₄Si) δ 14.39, 21.25, 34.27, 68.62, 121.01; ¹³C NMR (B; CDCl₃; Me₄Si) δ 13.77, 20.12, 33.96, 68.59, 120.80; molecular ion (A), calcd for C₄H₆NO m/z 84.0449, found M⁺ - CH₃ m/z 84.0454; molecular ion (B), calcd for C₄H₆NO m/z 84.0449, found M⁺ - CH₃ m/z 84.0440.

Compound C (peak C): IR ν_{\max} (neat, cm⁻¹) 3390, 2960, 2905, 2870, 2220, 1640, 1550, 1435, 1380, 1310, 1260, 1230, 1175, 1088, 1060, 1010, 935, 850, 740; MS, m/z 97 (M⁺, 23%), 82 (39), 68 (100), 67 (23), 54 (46), 52 (36), 41 (31); ¹H NMR (CDCl₃; Me₄Si) δ 1.95 (1 H, br s, OH), 2.04 (3 H, dt, J = 7.0 and 1.3 Hz, CH₃CH=C), 4.22 (2 H, dq, J = 1.3 and 1.3 Hz, HC=CCH₂OH), 6.53 (1 H, qt, J = 7.0 and 1.3 Hz, C=CH); ¹³C NMR (CDCl₃; Me₄Si) δ 17.00, 63.15, 115.84, 116.35, 144.13; molecular ion calcd for C₅H₇NO m/z 97.0528, found M⁺ m/z 97.0524.

Compound D (peak D): IR ν_{\max} (neat, cm⁻¹) 3400, 2920, 2235, 1640, 1555, 1440, 1385, 1240, 1180, 1040, 1000, 855, 765; MS, m/z 97 (M⁺, 5%), 82 (20), 79 (73), 68 (100), 54 (20), 52 (45), 41 (37); ¹H NMR (CDCl₃; Me₄Si) δ 1.90 (3 H, dt, J = 7.3 and 0.7 Hz, CH₃CH=C), 1.9 (1 H, br s, OH), 4.31 (2 H, dq, J = 1.0 and 0.7 Hz, HC=CCH₂OH), 6.59 (1 H, qt, J = 7.3 and 1.0 Hz, C=CH); ¹³C NMR (CDCl₃; Me₄Si) δ 14.59, 57.78, 115.72, 118.74, 145.31; molecular ion

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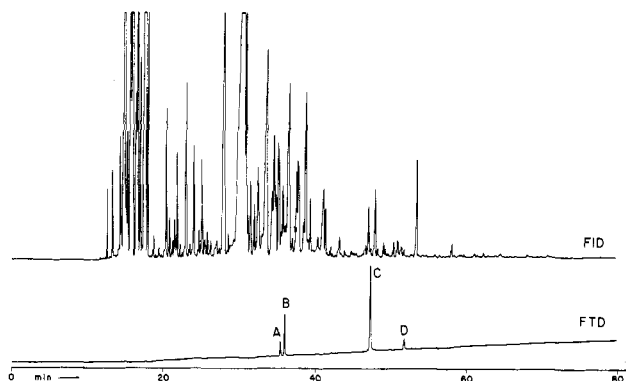


Figure 1. Gas chromatogram of the oil obtained from CH_2Cl_2 layer 1.

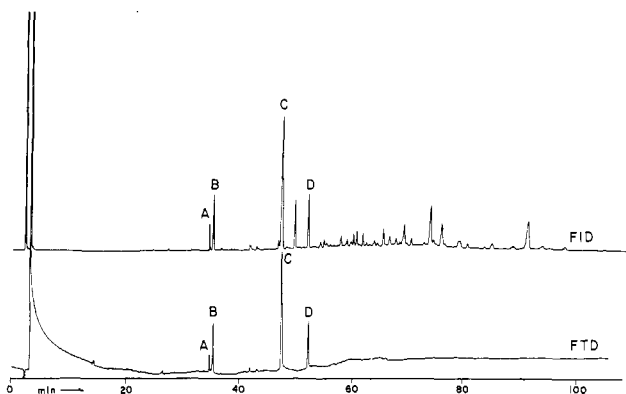


Figure 2. Gas chromatogram of the oil obtained from CH_2Cl_2 layer 2.

calcd for $\text{C}_5\text{H}_7\text{NO}$ m/z 97.0528, found M^+ m/z 97.0530.

Synthesis of 3-Hydroxy-2-methylbutyronitrile (1). NaBH_4 (0.36 g, 9.5 mmol) in limited amounts was added to a stirred cold solution of 3-chloro-2-butanone (3; 1 g, 9.4 mmol) in 10 mL of methanol. After completion of the addition, the reaction mixture was continuously stirred for 6 h. The solvent was removed with a rotary evaporator, and the residue was extracted with CH_2Cl_2 (10 mL) and washed with 3 mL of water. The extract was dried over Na_2SO_4 , and the solvent was removed (Pasto and Balasubramaniyam, 1967).

A mixture of the residue, H_2O (10 mL), NaCN (0.45 g, 9.2 mmol), and tetrabutylammonium bromide (0.5 g) was refluxed for 2 h (Starks, 1971). After the mixture was cooled to room temperature, the product was extracted with 5 mL of CH_2Cl_2 three times. The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated by evaporation. Subsequent preparative HPLC of the concentrated oil gave 3-hydroxy-2-methylbutyronitrile, **1a/1b** = ca. 1/1, 0.60 g (65%) (Asai, 1963; Goasdoué and Gaudemar, 1972).

RESULTS AND DISCUSSION

The gas chromatograms of CH_2Cl_2 layers 1 and 2 are shown in Figures 1 and 2, respectively. Figure 1 shows that small amounts of four nitrogen-containing compounds (peaks A–D) are present in CH_2Cl_2 layer 1. The GC pattern of blackcurrant buds absolute starting material was similar to that shown in Figure 1. Four nitrogen-containing compounds were not generated during the atmospheric steam distillation/extraction process but existed in blackcurrant buds absolute. On the other hand, these four nitrogen-containing compounds emerged as main components in CH_2Cl_2 layer 2 (Figure 2). Therefore, the concentrate of CH_2Cl_2 layer 2 was distilled under reduced pressure. The distillate was then subjected to preparative GLC to give a mixture of peaks A and B (A/B = ca. 1/2)

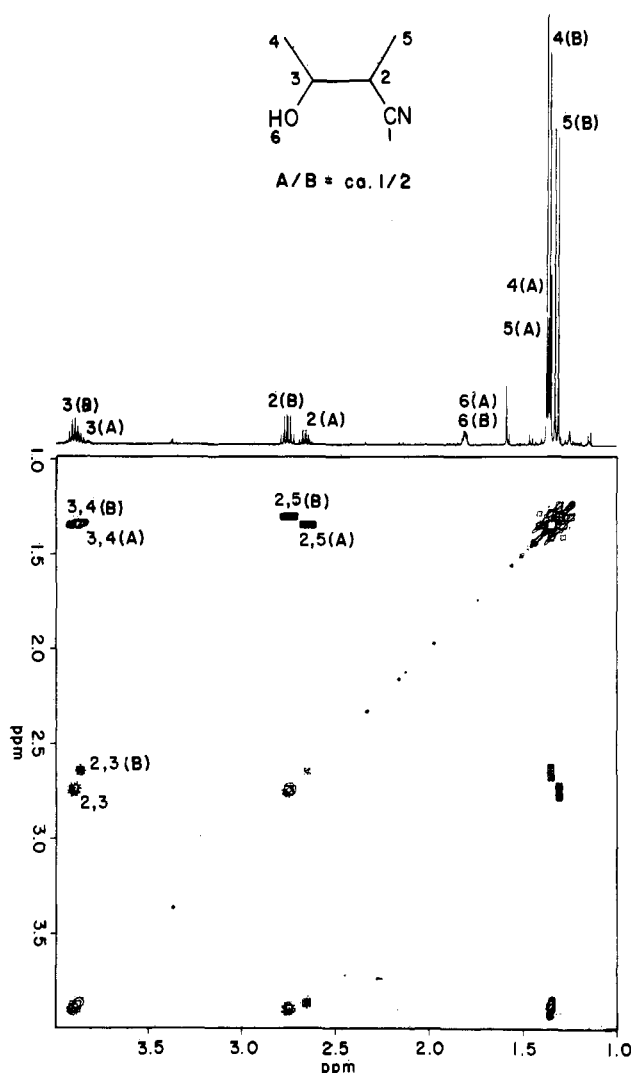
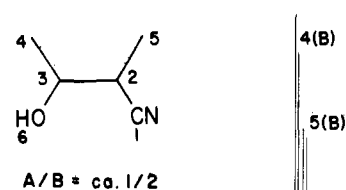


Figure 3. Two-dimensional ^1H NMR (COSY) spectrum of a mixture of compounds A and B (A/B = ca. 1/2; CDCl_3 ; 400 MHz, Bruker AM-400 spectrometer).

and peak C and peak D. Four nitrogen-containing compounds were tentatively named compounds A–D, respectively.

Compounds A and B (A/B = ca. 1/2). The mass spectra (EI, CI) of these compounds were very similar to each other. They had the molecular formula $\text{C}_5\text{H}_7\text{NO}$, established from HRMS and CIMS. The IR spectrum of a mixture of compounds A and B (A/B = ca. 1/2) showed characteristic absorptions at 3425 cm^{-1} (alcohol) and 2260 cm^{-1} (nitrile). The ^1H NMR spectrum of a mixture of compounds A and B showed the presence of two kinds of methyl groups adjacent to the nitrile groups, two kinds of methyl groups adjacent to the hydroxy groups, two kinds of methine groups attached to alcohols, and two kinds of methine groups attached to nitriles. In the two-dimensional NMR spectrum (Figure 3), coupling between individual protons was manifested by cross-peaks that appeared symmetrically with respect to the diagonal. The existence of two kinds of $\text{H}(5)\text{--H}(2)\text{--H}(3)\text{--H}(4)$ system was verified by cross-peaks $2,5(\text{A})/2,3(\text{A})/3,4(\text{A})$ and $2,5(\text{B})/2,3(\text{B})/3,4(\text{B})$. It was concluded from these spectral data that compounds A and B were the diastereomeric 3-hydroxy-2-methylbutyronitrile (**1a**, **1b**).

In order to confirm the structure of compound **1**, the compound was prepared from 3-chloro-2-butanone (**3**) via reduction and substitution. Synthetic and natural **1** had identical mass spectra and t_R (capillary GC).

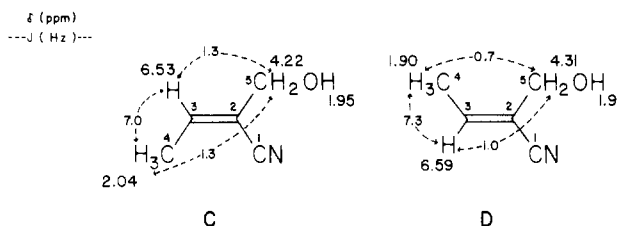


Figure 4. ^1H NMR data for (*Z*)- and (*E*)-2-(hydroxymethyl)-2-butenonitrile [(*Z*)-2, (*E*)-2].

Compounds C and D. The HRMS suggested the molecular formula of compound C to be $\text{C}_5\text{H}_7\text{NO}$. The IR spectrum of compound C indicated absorptions characteristic of alcohol (3390 cm^{-1}) and nitrile (2220 cm^{-1}). The ^1H NMR spectrum of compound C showed the presence of a hydroxy proton, a methyl group attached to an olefinic carbon, an olefinic proton, and a methylene proton adjacent to oxygen. The mass, IR, and ^1H NMR spectra of compound D were quite similar to those of compound C. It was concluded from these spectral data that compounds C and D were (*Z*) and (*E*) isomers of 2-(hydroxymethyl)-2-butenonitrile (2). In the ^1H NMR spectra, the chemical shift of the methylene proton adjacent to oxygen in compound D (4.31 ppm) was lower than that of compound C (4.22 ppm) due to the influence of the adjacent *cis* methyl group. The coupling constant values for $J_{4,5}$ were 1.3 and 0.7 Hz for compounds C and D, respectively. These ^1H NMR data indicate a *cis* configuration of C_4 and C_5 in compound D (Chamberlain, 1974). Accordingly,

compound C was determined as (*Z*)-2 and compound D as (*E*)-2 as shown in Figure 4 (Hearne and La France, 1952).

Compound 1 (diastereomers) has a faint odor. Compound (*Z*)-2 possesses a sweet and *p*-cresol derivative like odor, whereas compound (*E*)-2 has a sweet, powdery, and mild odor.

Registry No. 1 (isomer 1), 83968-05-2; 1 (isomer 2), 83968-04-1; (*E*)-2, 107407-88-5; (*Z*)-2, 107407-87-4.

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Model Reactions on Roast Aroma Formation. 1. Reaction of Serine and Threonine with Sucrose under the Conditions of Coffee Roasting and Identification of New Coffee Aroma Compounds

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The composition of volatiles from roasting mixtures of sucrose, serine, and threonine were analyzed by capillary GC/MS. About 350 compounds (alkyl-, alkenyl-, and acyl-substituted furans, pyrroles, pyrazines, pyridines, oxazoles, and others, among them nine pyrrolylalkanols) were identified. With the exception of furans and oxazoles, most of them were also formed after heating serine and threonine in the absence of sucrose. This reaction demonstrates the importance of pyrolytic reactions occurring in addition to the Maillard reaction. After roasting of green coffee, about 160 compounds were identified, 53 of which, up to now, have not been described as constituents of coffee aroma.

Roast aromas are well-known to consist of a great many compounds. For instance, more than 700 compounds have been reported in roast coffee aroma (Clifford, 1985). In the aromas of roasted meat or peanuts, about 500 and 300 compounds, respectively, have been identified at this time. Therefore, it is clear that the investigation of roast aromas is very difficult and time consuming. In the same manner, the analysis of one of the various roast aroma essences produced by the flavour industry is a very hard task.

These essences are mostly produced by heating of specially treated proteins with sugars, lipids, selected amino acids, and fruity acids. Short overviews are given by Baltes (1979, 1980). A great many aroma compounds formed by

these processes are of heterocyclic structure. They are formed by mechanisms that are postulated to involve the Maillard reaction (Baltes, 1980). But on the other hand, heating to about $250\text{ }^\circ\text{C}$ also enhances pyrolytic degradation.

We have been engaged for some time in investigating model reactions of roast aroma formation by treating one or two selected amino acids with sugars or sugar degradation products under food-processing conditions to study the compounds formed as well as their precursors.

Recently, we gave a report on our experiments on the reaction of serine and threonine with sucrose under the conditions of coffee roasting, because we were interested in the contribution of these compounds to coffee aroma (Baltes and Bochmann, 1986). In this paper we give more details about the experiments and some special compounds identified.

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