Essential Oils and Their Constituents. XXXIX.[1] Structures of Khusenic Acid and Isokhusenic Acid—Two Sesquiterpenic Constituents of Oil of Vetiver

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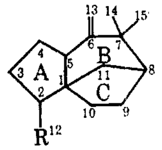
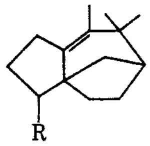
Khusenic acid and isokhusenic acid, two new sesquiterpenes isolated from oil of vetiver, are assigned structures 1 and 4 on the basis of experimental data obtained by degradation of their methyl esters.

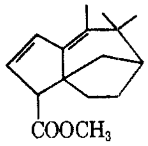
Khusenic and isokhusenic acid are two new sesquiterpenic constituents of oil of vetiver. A preliminary communication described the isomerization of the former into the latter in presence of mineral acid and pointed out the tricyclic character of these compounds.[3] Experimental results supporting structures 1 and 4 for khusenic and isokhusenic acids, respectively, have been presented.[4] A recent communication by Kido, *et al.*,[5] assigning structure 1 to zizanoic acid isolated from Japanese vetiver oil prompts the authors to publish the details of their investigations on khusenic acid and isokhusenic acid and to point out the possible identity of the former acid with zizanoic acid.



1, R = COOH
2, R = $COOCH_3$
3, R = CH_2OH



4, R = COOH
5, R = $COOCH_3$
6, R = CH_2OH



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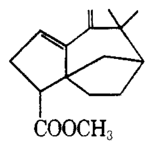

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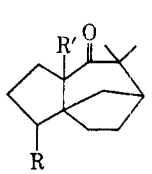
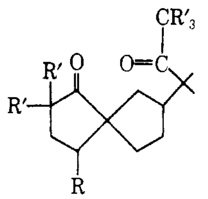
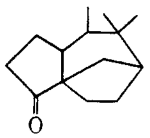


9, R = $COOCH_3$; R′ = H
10, R = CH_2OH; R′ = H
11, R = COOH; R′ = D



12, R = $COOCH_3$; R′ = H
13, R = COOH; R′ = H
14, R = CH_2OH; R′ = H
15, R = COOH; R′ = D



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A comparison of the reported physical properties of khusenic acid and zizanoic acid, and their methyl esters suggests that the two acids are the same compound.[3,5] Their identity is further borne out by the following results of degradations.[4,5] Barbier–Wieland degradation of dihydromethyl khusenate yielded a five-membered ring ketone 16 ($\nu_{\max}^{\mathrm{CCl_4}}$ 1736 cm^{-1}). The same ketone appears to have been obtained by Kido, *et al.*, by the degradation of dihydrozizanoic acid through the reaction sequence $>CHCOOH \rightarrow >CHCOCH_3 \rightarrow >CHOH \rightarrow >C{=}O$. On ozonolysis methyl khusenate gave a monoketo ester (9) (mp 102°, $\nu_{\max}^{\mathrm{CCl_4}}$ 1728, 1708 cm^{-1}), which yielded acid 11 on base-catalyzed deuteration, indicating that ring B was six- or seven-membered and that C-7 was either quaternary or was located at a bridgehead. Ozonolysis of methyl zizanoate and deuteration of the resulting monoketo ester (mp 103.5°, $\nu_{\max}^{\mathrm{CHCl_3}}$ 1723 and 1705 cm^{-1}) have also been reported to yield the same results.

The size of ring A was elucidated by the ozonolysis of methyl isokhusenate which yielded a diketo ester 12. The ir spectrum of 12 had a maximum at 1704 cm^{-1} which was assigned to the CH_3CO group. Absorption bands corresponding to the ester group and the second keto group were not resolved and appeared as a single intense peak at 1734 cm^{-1}. In order to clarify the nature of the second keto group, the diketo ester 12 was converted to the corresponding acid 13. A carbonyl band at 1737 cm^{-1} could now be detected and was assigned to a five-membered ring ketone. The absorption band corresponding to the CH_3CO group coalesced with that of the carboxyl carbonyl group appearing as a single peak at 1701 cm^{-1}. These spectral data suggested that ring A was five-membered.

The above evidence regarding the size of rings A and B was further supported by the results of ozonolysis of khusenol (3) and isokhusenol (6) obtained by reducing methyl khusenate and methyl isokhusenate, respectively, with lithium aluminum hydride. Khusenol yielded a monoketo alcohol (10) exhibiting the carbonyl absorption band at 1703 cm^{-1}, and isokhusenol gave a diketo alcohol (14) showing carbonyl absorptions at 1733 and 1701 cm^{-1}.

The treatment of methyl isokhusenate 5 with N-bromosuccinimide and subsequent dehydrobromination gave three products, two of which were the diene esters, 7 and 8. The third product could not be characterized. The uv spectrum of 7 exhibited a maximum at 253 $m\mu$ (ϵ 11,600), and its ir spectrum had absorption bands at 3065 (—CH=CH—), 1652, and 1622 cm^{-1} (conjugated double bonds). The nmr spectrum had signals at δ 6.43, 6.32 and 5.85, 5.77 for two olefinic protons, at 1.65 for a CH_3 group attached to an olefinic carbon atom, at 3.58 for the ester CH_3 group, and at 1.03 for two paraffinic CH_3 groups. On the basis of

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[1] Previous paper, *Indian Std. Inst. Bull.*, in press.

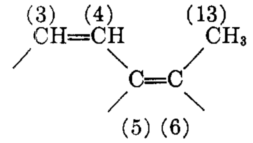
[2] National Research Council of Canada Postdoctorate Fellow, 1965–1967, on leave of absence from University of Hiroshima, Hiroshima, Japan.

[3] I. C. Nigam and H. Komae, *J. Pharm. Sci.*, **56**, 1299 (1967).

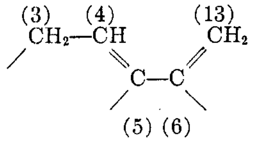
[4] H. Komae and I. C. Nigam, 153rd National Meeting of the American Chemical Society, Miami. Fla., April 1967, p O-60.

[5] F. Kido, H. Uda, and A. Yoshikoshi, *Tetrahedron Lett.*, 2815 (1967).

these spectral data the chromophoric group in the diene may be represented as



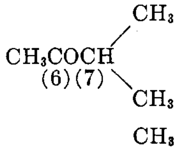
The second diene ester **8** exhibited uv absorption at 225 mμ (ϵ 5400) and its ir spectrum showed characteristic bands at 3081 and 3052 (=CH_2, =CH—), 1641 and 1620 (conjugated double bonds), 895 (>C=CH_2), and 840 cm^{-1} (>C=CH—). These assignments were consistent with the nmr spectrum of the diene, which had three olefinic proton signals at δ 4.70, 4.92, and 5.33, but no signal for a CH_3 group attached to olefinic C atom. The signals for the ester CH_3 and other two methyl groups now appeared at δ 3.65, 1.07, and 0.97, respectively. The disappearance of the CH_3—C= group and the simultaneous formation of a CH_2=C group suggested that the diene **8** was formed by isomerization of the diene **7** and the rearranged chromophoric group could be represented as



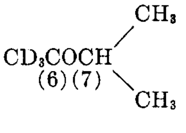
Base-catalyzed deuteration of the diketo ester **12** yielded mainly a pentadeuterated acid **15** ($C_{15}H_{17}D_5O_4$), thereby indicating the availability of five exchangeable protons on carbon atoms in α positions to the two keto groups. This observation, when considered along with the results of allylic dehydrogenation, confirmed the conclusions arrived at by deuteration of **9** and further emphasized the quaternary character of C-1.

The mass spectra of the acid **13** and its pentadeuterated derivative **15** exhibited strong peaks corresponding to stable molecular ions of mass number 86 and 89. The difference of three in the two mass numbers suggested that the fragments involved the methyl keto group and could be expressed as

m/e 86



m/e 89



These observations showed that C-7 carried two methyl groups and confirmed its quaternary nature, the ions *m/e* 86 and *m/e* 89 having been formed by β cleavage of the corresponding parent molecules.

The assignment of ring C^6 as a cyclopentane rather than a cyclobutane structure to ring C is preferred in view of the stability of this structure under varied and drastic dehydrogenation conditions.[3] Kido, *et al.*, have determined the mass spectra of three derivatives of zizanoic acid (= khusenic acid) and have found the spectral data consistent with their assigned structures.[5] The same inferences have been arrived at by the mass spectral analysis of several other compounds prepared in the authors' laboratory by the degradation of khusenic acid as well as isokhusenic acid.[7]

Experimental Section[8]

Ozonolysis of Methyl Isokhusenate.—Methyl isokhusenate (1.000 g) was dissolved in ethyl acetate (5 ml), cooled in an ice-salt freezing mixture, and ozonized using a Gallenkamp ozone apparatus GE-150. The reaction mixture was diluted with a further quantity of ethyl acetate (15 ml) and hydrogenated in the presence of 5% Pd-C catalyst at atmospheric pressure and at room temperature. The crude product (1.097 g) was chromatographed on silica gel (50 g) employing a mixture of benzene and ether (1:1) as eluent. Purified fractions (single peak on glpc analysis) were combined to get the diketo ester **12** (607 mg; $\nu_{max}^{CCl_4}$ 1733 and 1702 cm^{-1}; mol wt by mass spectrometry, 280).

Preparation of Diketo Acid 13.—A sample (32.4 mg) of the diketo ester **12** was refluxed for 30 min with a solution of 3.3 g of KOH in 9 ml of aqueous methanol (50%). The reaction mixture, on usual processing, gave an oily product (19.0 mg; $\nu_{max}^{CCl_4}$ 3500–2500, 1734, and 1701 cm^{-1}; mol wt by mass spectrometry, 266).

Preparation of Khusenol (3).—Lithium aluminum hydride (74.5 mg) in dry ether (5 ml) was added dropwise to an ice-cooled solution of methyl khusenate (490.0 mg) in dry ether (5 ml) with stirring. The mixture was refluxed under anhydrous conditions for 30 min and cooled. Excess hydride was decomposed by adding ethyl acetate (1 ml) followed by water (1 ml, dropwise). The ethereal layer was separated from the aqueous layer which was further extracted with three 2-ml portions of ether. The combined ether extracts were washed with water and dried. The residue (416.5 mg) obtained after removal of ether was chromatographed on alumina (grade II, 15 g) employing hexane (four 20-ml portions), hexane–benzene (1:1 mixture, four 20-ml portions), benzene (five 20-ml portions), and benzene–ether (1:1 mixture, seven 20-ml portions). Fractions obtained with benzene (340.1 mg) showed identical ir spectra, the absorption bands being at 3632, 3085, 1788, 1634, 1458, 1386, 1368, 1178, 1166, 1121, 1074, 1052, 1022, 945, 982, 925, and 891 cm^{-1}. Retention time was 3.6 min on SE-30 column at 250°.[3]

The 3,5-dinitrobenzoate had mp 117°. *Anal.* Calcd for $C_{22}H_{26}O_6N_2$: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.33; H, 6.31; N, 6.75; mol wt of khusenol (mass spectrometry), 220.

Ozonolysis of Khusenol.—Khusenol (290.5 mg) in CCl_4 (5 ml) solution was ozonized as described above for methyl isokhusenate. The resulting ozonide (359.0 mg) was decomposed by heating with water (5 ml), cooled, and extracted with ether. The aqueous layer, on treatment with a saturated solution of dimedone, yielded a crystalline derivative (mp 187°, no depression on admixture with authentic formaldehyde–dimedone derivative). The ether extract was dried and evaporated, and the residue was freed from acidic products by treatment with Amberlite IRA-400 (2 g) by the method described before. The neutral product was chromatographed on alumina (grade II, 10 g) and eluted successively with hexane, benzene, benzene–ether (1:1), and ether. The later hexane fractions, all the benzene fractions, and early benzene–ether fractions were chiefly composed of the same compound (glpc and infrared spectra). Repeated chromatography yielded a pure sample (retention time on SE-30 column at 250°, 4.4 min; ν_{max}^{liq} 3630, 1703, and 1022 cm^{-1}).

Preparation and Ozonolysis of Isokhusenol (6).—Lithium aluminum hydride reduction of methyl isokhusenate (1130.5 mg) according to the procedure adopted for the preparation of khusenol yielded crude isokhusenol (889.5 mg). The product was chromatographed on alumina (grade II, 20 g) using hexane,

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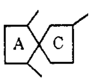
(6) Dr. Klein of Dragoco, Holzminden, West Germany, has kindly informed the authors that his group has verified the size of ring C and the location of the carboxyl group by degradation of the acid to the spiro hydrocarbon



(7) I. C. Nigam and J. L. Holmes, unpublished data.

(8) Melting points are uncorrected. Apparatus and other pertinent details for uv, ir, nmr, and mass spectral measurements, microanalysis and gas chromatographic determinations were described before.[3]

hexane–benzene (1:1), benzene, and ether as eluents. The hexane–benzene mixture and benzene gave isokhusenol-enriched fractions (684.1 mg; purity 90–95%; retention time on SE-30 column, 3.1 min; $\nu_{\max}^{\text{liq}}$ 3634, 1085, 1070, 1038, and 980 cm^{-1}). Molecular weight (mass spectrometry) of a sample purified by glpc was 220.

A portion (351.9 mg) of the above sample of isokhusenol was ozonized as described under "Ozonolysis of Khusenol." The resulting neutral product (235.1 mg) was chromatographed on alumina (grade III, 10 g), using hexane, benzene, and benzene–ether (1:1) successively as eluents. The last solvent eluted homogeneous fractions which on further chromatography yielded a practically pure sample (retention time on SE-30 column at 250°, 6.6 min; $\nu_{\max}^{\text{liq}}$ 3630, 1733, and 1701 cm^{-1}).

Deuteration of the Monoketo Ester 9.—A sample (24.6 mg) of the monoketo ester **9**, prepared by ozonization of methyl khusenate as described before,[3] was added to a solution of sodium deuteroxide obtained by reacting sodium metal (125 mg) with D_2O (1 ml). The reaction mixture was refluxed for 24 hr, cooled, acidified with DCl, and extracted with three 10-ml portions of ether. The ether extract was dried and evaporated under reduced pressure. The residue was dissolved in ethanol (2 ml) and the solvent removed under reduced pressure. The treatment with ethanol was repeated twice. Mass spectral analysis of the final product (20.3 mg) showed the parent peak at *m/e* 237, corresponding to the parent molecular ion of the monodeuterio acid **11**. Redeuteration of the product did not produce any change in the mass spectrum.

Deuteration of the Diketo Ester 12.—Diketo ester **12** (21.6 mg) was subjected to deuteration as described above for the monoketo ester **9**. The resulting product (21.5 mg) on mass spectral analysis was found to be a mixture of deuterio derivatives of the diketo acid **13**, the peak possessing the highest *m/e* value (271) corresponded to pentadeuterated acid.

Reaction of Methyl Isokhusenate with N-Bromosuccinimide.—Methyl isokhusenate (201 mg), dry carbon tetrachloride (3 ml), and N-bromosuccinimide (147 mg) were heated under reflux by an infrared lamp for 3 hr. The reaction mixture was cooled and filtered. The residue obtained by evaporation of the filtrate was boiled with potassium acetate (2 g) in acetic acid (8 ml) for 40 min. After cooling, the reaction mixture was poured into a separatory funnel containing NaOH (5 g) and ice–water (250 g), and extracted with four 50-ml portions of ether. The ether extract was freed from alkali by washing with water, then dried and evaporated. The resulting product (176 mg) was chromatographed on silica gel (13 g). Elution with a mixture of benzene and ether (4:1) gave four fractions (8 ml each) yielding products weighing 122, 18, 7, and 3 mg, successively. The first fraction was resolved into four constituents by gas chromatography.[9] These were characterized as follows. **Peak No. 1** (60%, retention time 7.0 min): unchanged methyl isokhusenate. **Peak No. 2** (13%, retention time 8.1 min): $\lambda_{\max}$ 225 mμ (ϵ 5400); $\nu_{\max}^{\text{CCl}_4}$ 3081, 3052, 1735, 1641, 1620, 895, and 840 cm^{-1}; nmr peaks at δ 5.33 (H), 4.92 and 4.70 (2H), 3.65 (3H), and 1.07 and 0.97 (6H). **Peak No. 3** (16%, retention time 9.8 min): $\lambda_{\max}$ 253 mμ (ϵ 11,600); $\nu_{\max}^{\text{CCl}_4}$ 3065, 1734, 1652, and 1622 cm^{-1}; nmr peaks at δ 6.43 and 6.32 (H), 5.85 and 5.77 (H), 3.58 (3H), and 1.03 (6H). **Peak No. 4** (11%, retention time 12.0 min): $\lambda_{\max}$ 320 mμ (ϵ 7200).

Preparation of Ketone 16.—Hydrogenated methyl khusenate (100 mg), prepared as described before,[3] was dissolved in ether (1 ml) and added dropwise to an ice-cooled solution of Grignard reagent prepared from magnesium turnings (34 mg), bromobenzene (225 μl), and ether (1 ml). The reaction mixture was refluxed for 2 hr and then left overnight at room temperature. The excess reagent was decomposed with saturated solution of ammonium chloride (4 ml) and the mixture, after dilution with water, was extracted with three 10-ml portions of ether. The crude product (185 mg), recovered by evaporating the dried extract, was chromatographed on grade II alumina (5 g) and eluted successively with 10 ml each of (i) hexane, (ii) hexane–benzene (1:1), (iii) benzene, (iv) benzene–ether (1:1), and (v) ether. Fractions ii–iv, found by ir analysis to be rich in the diphenyl carbinol, were combined (76 mg) and refluxed with formic acid (5 ml) for 6 hr. The reaction mixture was diluted with water and extracted with three 10-ml portions of ether. The crude product (59 mg), obtained by evaporation of the dried ether extract, was chromatographed on grade I alumina (4 g). Hexane (30 ml) eluted a fraction (32 mg) rich in diphenyl olefin, which was ozonized; the reaction product was worked up as described above for khusenol. The product of ozonolysis (33 mg) was repeatedly chromatographed on grade II alumina employing hexane as eluent to give a fraction rich in ketone **16** ($\nu_{\text{C=O}}$ 1736 cm^{-1}; parent peak in the mass spectrum at *m/e* 206).

Registry No.—**1,** 16203-25-1; **3,** 16223-63-5; **3** 3,5-dinitrobenzoate, 16202-78-1; **4,** 16202-79-2; **6,** 16202-80-5; **7,** 16202-81-6; **8,** 16202-82-7; **9,** 16202-83-8; **10,** 16202-85-0; **12,** 16202-84-9; **13,** 16202-86-1; **14,** 16202-87-2; **16,** 16202-88-3.

Acknowledgments.—The authors are grateful to Dr. Leo Levi for his interest in these investigations, and to Dr. G. A. Neville and Dr. J. L. Holmes for the measurement of nmr spectra and mass spectra, respectively.

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[9] Gas chromatographic analysis on 20% Reoplex 400 column at 220°. For details of instrument, etc., see I. C. Nigam and L. Levi, *Can. J. Chem.*, **40**, 2083 (1962).