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MONOTERPENE ALDEHYDES AND ISOPHORONE-RELATED COMPOUNDS OF SAFFRON

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Abstract—The volatile constituents of saffron were extracted with diethyl ether, and the individual fractions were separated by gas chromatography. IR, UV and NMR spectroscopy and mass spectrometry were used to identify these previously unreported compounds in saffron: 2,6,6-trimethyl-4-hydroxy-1-cyclohexen-1-carboxaldehyde, 2,4,4-trimethyl-3-formyl-6-hydroxy-2,5-cylohexadien-1-one, isophorone, 3,5,5-trimethyl-4-hydroxy-1-cyclohexanon-2-ene, 3,5,5-trimethyl-1,4-cyclohexadione, 3,5,5-trimethyl-1,4-cyclohexadion-2-ene, and 3,5,5-trimethyl-2-hydroxy-1,4-cyclohexadion-2-ene. The last four of these compounds were synthesized from isophorone.

INTRODUCTION

SAFFRON, a spice composed of the dried stigmas of *Crocus sativus* L., is used for flavoring and coloring food preparations. Despite its use for many centuries, very little is known about its volatile constituents. Previous to the present study, the only compound identified in the volatiles of this spice was safranal.[1] This aldehyde has been synthesized by Kuhn and Wendt[2] and more recently by Mousseron-Canet *et al.*[3]

This paper describes the identification of seven previously unreported volatile constituents of saffron as isophorone, 3,5,5-trimethyl-4-hydroxy-1-cyclohexanon-2-ene (I), 3,5,5-trimethyl-1,4-cyclohexadione (II), 3,5,5-trimethyl-1,4-cyclohexadion-2-ene (III), 3,5,5-trimethyl-2-hydroxy-1,4-cyclohexadion-2-ene (IV), 2,6,6-trimethyl-4-hydroxy-1-cyclohexene-1-carboxaldehyde (V), and 2,4,4-trimethyl-3-formyl-6-hydroxy-2,5-cyclohexadien-1-one (VI).

RESULTS

The major volatile constituent of saffron was identified as safranal by comparison of its NMR spectrum with that reported by Mousseron-Canet *et al.*[3] Another volatile fraction, isophorone, was identified by direct comparison of its IR, UV, mass spectra, and GLC retention time with those of the authentic sample.

Compound I had IR absorptions at 3420 (hydroxyl) and 1665 cm^{-1} (conjugated carbonyl). The accurate mass determination revealed a parent peak at *m/e* 154·1041, dictating a molecular formula of $C_9H_{14}O_2$, and other fragments at *m/e* 112 (M^+-42) and 98 (M^+-56). Signals in the NMR spectrum at 1·01 (3H, singlet), 1·08 (3H, singlet), 1·25 (1H, broad), 2·03 (3H, doublet, J = 1·0 Hz), 2·18 (1H, singlet), 2·23 (1H, singlet), 3·95 (1H, broad), and 5·75 (1H, broad) were consistent with the structure proposed. This structure was further substantiated by its synthesis from isophorone. (Scheme A).

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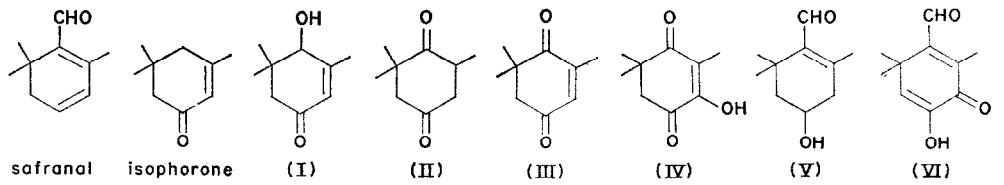
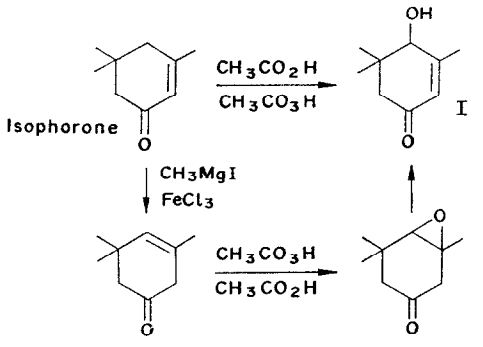
[1] R. KUHN and A. WINTERSTEIN, *Chem. Ber.* **67**, 344 (1934).
[2] R. KUHN and G. WENDT, *Chem. Ber.* **69**, 1549 (1936).
[3] M. MOUSSERON-CANET, J. C. MANI and J. L. OLIVE, *C.R. Hebd. Acad. Sci. Paris Series* **262C**, 1725 (1966).

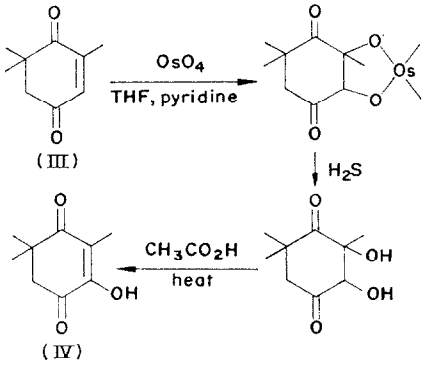


3,5,5-Trimethyl-1,4-cyclohexadione (II) had an IR absorption at 1710 cm^{-1} (non-conjugated carbonyl). The NMR spectrum had signals for nine protons at δ1·06 and 1·10, and several other signals (5H) between 2·00 and 3·30 ppm. The mass spectrum had a parent peak at *m/e* 154·1036, indicating a molecular formula of $C_9H_{14}O_2$, and other fragments at *m/e* 139 (M^+-15) and 112 (M^+-42). There was no absorption in the UV, which is consistent with that of the NMR and IR spectra. Also, the compound did not change when refluxed in 1 N H_2SO_4 at 90° for 24 hr. Finally, the assigned structure II was confirmed by its synthesis from the rearrangement of compound I. This rearrangement was carried out in benzene containing catalytic amounts of *p*-toluene sulfonic acid.[4]

3,5,5-Trimethyl-1,4-cyclohexadion-2-ene (III) had a UV absorption with λ_{max} at 240 nm. The IR spectrum had absorptions at 1685 and 1625 cm^{-1}, indicating the presence of a conjugated carbonyl. In the mass spectrum there was a parent peak at *m/e* 152·0837, assigning a molecular formula of $C_9H_{12}O_2$, and other fragments at *m/e* 137 (M^+-15) and 96 (M^+-56). The NMR spectrum has signals at δ1·21 (6H, singlet), 1·96 (3H, doublet, J = 1·0 Hz), 2·61 (2H, singlet), and 6·46 (1H, multiplet). The proposed structure III was confirmed by its synthesis from the oxidation of I.



SCHEME A. SYNTHESIS OF I FROM ISOPHORONE.



SCHEME B. SYNTHESIS OF COMPOUND IV FROM III.

A solid, m.p. 135–136°, isolated from saffron had IR absorptions at 3378 cm^{-1}, indicating the presence of a hydrogen-bonded hydroxyl, and at 1680, 1658, and 1640 cm^{-1}, indicating the presence of a conjugated carbonyl. Other absorptions were also observed at 2980, 1465, 1385, 1360, 1300, 1205, 1165, 1121, and 1060 cm^{-1}. The mass spectrum (Table 1) had a parent peak at *m/e* 168·0755, dictating a molecular formula of $C_9H_{12}O_3$. Other fragments at *m/e* 153 (M^+-15) and 140 (M^+-28) indicated the presence of methyl and carbonyl groups, respectively. The UV spectrum had a λ_{max} at 288 nm an indication of the presence of an α-hydroxy enone. The NMR spectrum had signals at δ1·25 (6H, singlet), 1·95 (3H, singlet),

[4] J. N. MARX and F. SONDHEIMER, *Tetrahedron* Suppl. 8, Part 1 (1966).

TABLE 1. THE MASS SPECTRUM OF 3,5,5-TRIMETHYL-2-HYDROXY-1,4-CYCLOHEXADION-2-ENE

m/e	Abundance	*m/e*	Abundance
18	34·3	77	1·4
27	30·1	80	4·1
28	4·1	82	11·0
29	20·5	83	57·6
39	35·6	84	100
40	1·4	85	39·7
41	34·3	107	5·5
42	1·4	112	2·7
43	16·4	125	57·6
53	6·8	126	64·4
55	46·6	127	2·7
56	75·4	140	31·5
57	48·0	153	71·3
67	1·4	154	1·4
69	43·8	168	72·6 M^+
		169	6·8

2·70 (2H, singlet), and 6·80 (1H, singlet) assigned to a *gem*-dimethyl group, vinylic methyl, methylene, and an acidic hydroxyl proton, respectively. These assignments are consistent with those based on the IR, UV and mass spectra. The structure IV assigned to this compound was confirmed by synthesis from III (Scheme B).

Compound V was isolated by GLC techniques from an ether extract of the saffron, using an OV-210 glass column. This constituent was further purified on a SF96 (50) glass column, but was found not to elute from stainless steel columns. The mass spectrum of compound V (Table 2) had a molecular ion at *m/e* 168·1139, assigning a formula of $C_{10}H_{16}O_2$. Fragments were observed at *m/e* 153 (M-15) indicating the loss of a methyl radical, and at *m/e* 150 (M-18), indicating the loss of water. The aldehyde function of compound I was indicated by the fragment at *m/e* 139 (M-29). A base peak, *m/e* 135 (M-15-18), resulted from the loss of both water and a methyl fragment.

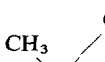
TABLE 2. MASS SPECTRA* OF 2,6,6-TRIMETHYL-4-HYDROXY-1-CYCLOHEXEN-1-CARBOXALDEHYDE (V) AND 2,4,4-TRIMETHYL-3-FORMYL-6-HYDROXY-2,5-CYCLOHEXADIEN-1-ONE (VI)

	Relative abundance			Relative abundance			Relative abundance			Relative abundance	
m/e	V	VI	*m/e*	V	VI	*m/e*	V	VI	*m/e*	V	VI
30	68	69	79	60	39	121	70	—	150	29	—
41	61	33	80	13	6	122	33	—	151	—	41
43	61	27	81	56	24	123	18	76	152	—	43
51	23	33	91	67	35	124	—	49	153	52	2
53	52	37	93	24	4	125	12	—	165	—	33
55	61	24	95	26	14	135	100	6	168	68	—
65	24	20	105	34	4	136	15	—	180	—	98
67	31	14	107	96	24	137	—	88			
69	35	25	108	20	14	138	—	37			
77	48	49	109	50	100	139	12	—			

* Prominent mass spectrum fragments (when *m/e* ≥ 12 on either one of the spectrum) are listed.

The IR spectrum of compound V had absorptions at 3400 and 1075 cm^{-1} (hydroxyl), 2870, 2765, and 1665 cm^{-1} (an indication of a conjugated aldehyde moiety), and at 1615 cm^{-1} (unsaturation). A doublet centered at 1375 cm^{-1} indicated the presence of a *gem*-dimethyl group. The UV spectrum had an absorption with λ_{max} at 245 nm, which is consistent with the presence of a trisubstituted, conjugated aldehyde. The NMR spectrum (Table 3) verified the aldehyde function (δ10·1, 1H as a sharp singlet), the *gem*-dimethyl group (δ1·25, 6H, singlet), and the hydroxyl function (δ3·00, 1H, broad singlet). The NMR

TABLE 3. SIGNALS IN THE NMR SPECTRA OF 2,6,6-TRIMETHYL-4-HYDROXY-1-CYCLOHEXEN-1-CARBOXALDEHYDE (V) AND 2,4,4-TRIMETHYL-3-FORMYL-6-HYDROXY-2,5-CYCLOHEXADIEN-1-ONE (VI)

Compound				—O—H	Other
V	1·25s	2·15s	10·10s	3·00s	1·55m*, 2·40m†, 3·85m‡
VI	1·41s	2·33s	10·30s	5·98s	6·05s§

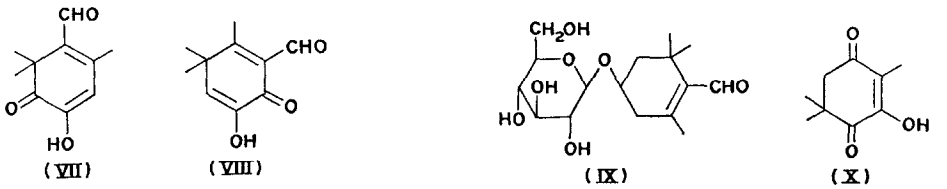
* 2H on C-5.
† 2H on C-3.
‡ CH—O of secondary alcohol.
§ Vinyl proton.

spectrum further indicated a vinyl methyl group (δ2·15, 3H, singlet). A multiplet at δ3·85 (1H) is interpreted as the *CH-O* proton of a secondary alcohol coupling with four dissimilar protons. Additional signals were centred at δ1·55 and 2·40, account for these remaining 4-ring protons. Compound V was converted to safranal in acid. Compound VI also did not elute from stainless steel GLC columns. However, this constituent was isolated pure from an ether extract of saffron by using glass column GLC techniques with OV-210 and FFAP, respectively, as liquid phases. Compound VI, a yellow solid, m.p. 107–108°, was assigned a molecular formula of $C_{10}H_{12}O_3$ based on the molecular ion at *m/e* 180·0814 in its mass spectrum. Compound VI had an IR absorption (KBr) at 3400 cm^{-1} as a sharp spike, indicating the presence of an internally hydrogen-bonded hydroxyl group. Two strong absorptions at 1685 and 1630 cm^{-1} indicated the presence of at least one conjugated carbonyl. Other bonds occurred in the IR spectrum at 1435, 1380, 1312, 1260, 1118, 1020, 885 and 790 cm^{-1}. The NMR spectrum of compound VI was similar to that of compound V (Table 3). The sharp singlets in the NMR support the assignments of a conjugated aldehyde (δ10·3, 1H), a *gem*-dimethyl group (δ1·41, 6H), a vinyl methyl (δ2·33, 3H) and an isolated vinyl proton (δ6·05, 1H). The hydroxy proton was observed to be at δ5·89 (1H, singlet) as the chemical shift of this acidic proton in the NMR spectrum determined in pyridine (d_5) was altered. All the prominent fragments of less than *m/e* 121 in the mass spectrum of compound V were also observed in the mass spectrum of compound VI (Table 2). Of the larger fragments in the mass spectrum of compound VI, those at *m/e* 165 (M-15), 152 (M-28), and 151 (M-29) account for the losses of a methyl radical, carbon monoxide, and the aldehyde function, respectively. The base fragment, *m/e* 109, can be accounted for by the loss of two carbon monoxide functions and the subsequent loss of a methyl radical.

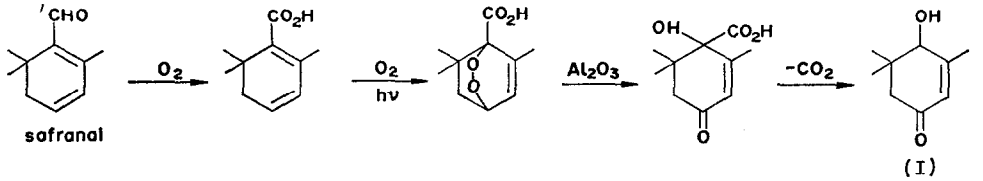
The observed UV absorption at 266 nm supports a structural assignment as VI and does not permit assigned structures which contain triconjugated or cisoid moieties as in structure VII. Further, the NMR spectrum of compound VI, using a 100 megacycle spectrometer, did not show any splitting of the bands representing the vinyl methyl or the vinyl proton moieties. This indicates the vinyl methyl and the vinyl proton were not on adjacent carbon atoms. Possible structures which do not conform to the isoprene rules have not been considered, for obvious reasons. However, structure VIII, which does fit the isoprene rules, seems less likely than the assigned structure VI, because of the positions of oxidation. The similarities in the mass spectra of compounds V and VI and the fact that the aldehyde functions of picrocrocin, safranal, and compound V all occur on the same carbon, suggest similar positions of oxidation, structure (VI), for this new aldehyde.

DISCUSSION

Table 4 lists, with respect to safranal, the relative concentrations of the previously unreported volatile constituents isolated from saffron. Isophorone and the compounds I, II, III, and IV all possess nine carbons. In contrast, the only previously reported volatile constituent, safranal, is a terpene aldehyde, as are compounds V and VI. Hydrolysis of picrocrocin (IX), a bitter constituent reported in saffron,[1] may account for the origin of both compound V and safranal, and subsequent oxidation could lead to compound VI.



Isophorone, although not commonly found in plants, has been isolated from the volatiles of cranberries (*Vaccinium macrocarpon* Ait).[5] Compounds I, II, and III have not been found in any natural product although they have been synthesized.[4,6] However, compound IV has neither been reported in any natural product, nor has it previously been synthesized. Schamp[7] has reported the synthesis of a similar compound (X).



SCHEME C. OXIDATION OF SAFRANAL IN UV LIGHT.

To determine if safranal could be the precursor of these isophorone-related compounds, a sample of safranal was irradiated with long wavelength UV light and the absorption (λ_{max} at 310 nm) was recorded at 5 min intervals. At 20 min the absorption at 310 nm had decreased and a new peak with λ_{max} at 233 nm had appeared. The predicted reaction sequence (Scheme C) is supported by the work of Mousseron-Canet *et al.*[3]

[5] K. ANJOU and E. VON SYDOW, *Acta Chem. Scand.* **21**, 2076 (1967).
[6] H. MAYER, M. MONTAVON, R. RUEGG, and O. ISLER, *Helv. Chim. Acta* **50**, 1606 (1967).
[7] N. SCHAMP, *Verh. Kom. Vlaam, Acad. Wetensch Belg. Kl. Wetensch.* **28**, 90 (1966).

TABLE 4. THE RELATIVE CONCENTRATIONS OF THE VOLATILE CONSTITUENTS OF SAFFRON WITH RESPECT TO SAFRANAL

Compound	Relative concentration
Safranal	100
Isophorone	3·94
3,5,5-trimethyl-4-hydroxy-1-cyclohexanon-2-ene (I)	12·80
3,5,5-trimethyl-1,4-cyclohexadione (II)	3·28
3,5,5-trimethyl-1,4-cyclohexadion-2-ene (III)	2·47
3,5,5-trimethyl-2-hydroxy-1,4-cyclohexadion-2-ene (IV)	2·47
2,6,6-trimethyl-4-hydroxy-1-cyclohexen-1-carboxaldehyde (V)	29·40
2,4,4-trimethyl-3-formyl-6-hydroxy-2,5-cyclohexadien-1-one (VI)	12·80

Gas chromatographic peak areas were used to estimate the composition without correction factors.

As evidenced by the above equations, the isophorone-related compounds may be nonenzymatically formed by the oxidation and decarboxylation of safranal followed by the oxidation and isomerization of I. However, because both the oxidized and the reduced isophorone-related compounds are present in saffron, they are believed to be formed enzymatically.

Saffron is known to have antibacterial and antiviral activities.[8] Tiffany *et al.*[9] and Myrvik *et al.*[10] have demonstrated such activities by 6-membered ring compounds which have 1,2-dione moieties. On this basis, compounds IV and VI, which possess a 1,2-dione moiety, may contribute to these physiological activities of saffron.

EXPERIMENTAL

IR spectra were measured on a Perkin-Elmer 257 spectrophotometer as thin films or in KBr pellets, and NMR spectra were measured on a Varian A-60 instrument with TMS as the internal standard. The UV spectra were measured in methanol on a Beckman DB-G spectrophotometer, and the mass spectra on a Varian M66 spectrometer.

Extraction and Isolation of the Volatile Constituents

The lipid constituents of saffron were extracted with Et_2O in a dry ice bath. After centrifugation and filtration, the extract was concentrated and the volatiles were separated and recovered using gas chromatographic techniques. The chromatograph was equipped with both an OV 210 and a FFAP glass column. The fractions were collected as described by Jennings *et al.*[11] and characterized using spectral methods as described by Varo and Heinz.[12]

Synthesis of Isophorone-related Compounds

3,5,5-*Trimethyl*-4-*hydroxy*-1-*cyclohexanon*-2-*ene*(I). 3,5,5-Trimethyl-1-cyclohexanon-3-ene[13](1·45 m-moles) was added to 10·5 m-moles of peracetic acid in 1·6 ml HOAc, and kept at 4° overnight. The pH was adjusted to 8 with 40% NaOH. 10 ml H_2O were added and the mixture was extracted twice with 25 ml Et_2O. The

[8] C. L. MADAN, B. M. KAPUR and U. S. GUPTA, *Economic Botany* **20,** 377 (1966).
[9] D. TIFFANY, J. B. WRIGHT, R. B. MOFFETT, R. V. HEINZELMAN, R. E. STRUBE, B. D. ASPERGREN, E. H. LINCOLN and J. L. WHITE, *J. Am. Chem. Soc.* **79,** 1682 (1957).
[10] N. Q. MYRVIK and W. A. VOLK, *J Bacteriol.* **68,** 622 (1954).
[11] W. G. JENNINGS, R. R. CREVELING and D. E. HEINZ, *J. Food Sci.* **29,** 730 (1964).
[12] P. T. VARO and D. E. HEINZ, *J. Agr. Food Chem.* **18,** 234 (1970).
[13] M. S. KHARASCH and P. O. TAWNEY, *J. Am. Chem. Soc.* **63,** 2308 (1941).

layers were combined, washed with 20 ml saturated NH_4Cl, and then washed twice with 25 ml H_2O. The Et_2O was dried (Na_2SO_4) and the solvent removed under a stream of N_2. Compound I was isolated from a mixture of more than ten products using GLC techniques and was identical (IR, UV and NMR) with the natural product.

3,5,5-*Trimethyl*-1,4-*cyclohexadione* (II). 3,5,5-Trimethyl-4-hydroxy-1-cyclohexanon-2-ene (0·0195 mmole) was reacted with *p*-toluene sulfonic acid (0·00526 m-moles) in 100 μl of benzene at 90° for 16 hr. The GLC analysis revealed only one product, and that was identical with compound II (IR).

3,5,5-*Trimethyl*-1,4-*cyclohexadion*-2-*ene* (III). CrO_3 (0·7 mmole) in 200 μl of water was added to compound I (1 m-mole) in 200 μl HOAc and 500 μl H_2O. The solution was shaken at 26° for 2 hr and then mixed with 20 ml H_2O and 10 ml Et_2O. The Et_2O was washed with 20 ml of saturated NH_4Cl and 2 × 20 ml H_2O. After drying (Na_2SO_4) the Et_2O was evaporated. Compound III was isolated by GLC techniques and was identical (IR) with the natural product.

3,5,5-*Trimethyl*-2-*hydroxy*-1,4-*cyclohexadion*-2-*ene* (IV). Compound III (0·066 m-moles) in 100 μl of pyridine was mixed with 0·066 mmoles of OsO_4 in 50 μl of THF. The mixture was then left at 4° overnight. H_2S was bubbled into the tube which caused a black precipitate to form. 500 μl HOAc were added, and the mixture was left at 26° for 20 min. The mixture was filtered and washed with 20 ml of $CHCl_3$–CH_3OH (50:50 v/v). The filtrate was placed in an oil bath at 145° for 20 min, during which time the $CHCl_3$ and MeOH mostly evaporated from the solution.

The remaining acetic acid was transferred to a separatory funnel containing 20 ml each H_2O and Et_2O. The mixture was shaken vigorously for 2 min, after which the Et_2O removed, further washed with 20 ml H_2O, and dried (Na_2SO_4). Compound IV was isolated by GLC techniques and was identical (m.p., IR, UV) to the natural product.

Hydrolysis of Compound V

The addition of acetic acid to compound V dissolved in CCl_4 yielded safranal, identified by its retention time on GLC and its IR and UV spectra.

Acknowledgements—We wish to thank the McCormick Company for the generous supply of saffron and K. Miyano for the mass spectra.