# 3,6-NONADIEN-1-OL FROM CITRULLUS VULGARIS AND CUCUMIS MELO

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Abstract—cis,cis-3,6-Nonadien-1-ol isolated from watermelon and previously from muskmelon by vacuum steam distillation-extraction, was identified by interpretation of spectra and by comparison with synthetic *trans,cis*-3,6-nonadien-1-ol. The flavor of *cis,cis*-3,6-nonadien-1-ol was described by flavor panel members as reminiscent of watermelon or watermelon rind and the threshold concentration of the compound in aqueous solution was estimated at 10 ppb. Possible precursors for the C<sub>9</sub> aldehydes and alcohols isolated from melon are presented.

### INTRODUCTION

EARLIER we reported the isolation from muskmelon fruit essence of several  $C_9$  aldehydes and alcohols including a nonadienol. The present work was undertaken primarily to complete identification of the nonadienol and in addition to obtain information on the flavor quality and threshold of this compound.

## **RESULTS AND DISCUSSION**

The melon component was isolated by vacuum steam distillation-extraction and was purified by GLC. The MS of the compound yielded diagnostic peaks at m/e 31 (CH<sub>2</sub>OH), m/e 122 (M-H<sub>2</sub>O) and a low intensity M<sup>+</sup> at m/e 140. The overall fragmentation pattern was similar to that of other nonadienols.<sup>1</sup> An NMR spectrum showed the following resonances:  $3.68 \delta$  (2H,t,-CH<sub>2</sub>-CH<sub>2</sub>-OH),  $2.37 \delta$  (2H,q, =CH-CH<sub>2</sub>-CH<sub>2</sub>-OH),  $2.83 \delta$  (2H,t,=CH-CH<sub>2</sub>-CH=),  $2.08 \delta$  (2H,p, Me-CH<sub>2</sub>-CH=),  $0.98 \delta$  (3H,t, CH<sub>3</sub>-CH<sub>2</sub>-CH=), and  $5.4 \delta$  (4H,m,-CH=CH-CH<sub>2</sub>-CH=CH-). From these data it was concluded that the nonadienol double bonds are located at C-3 and C-6. An IR spectrum obtained earlier using CCl<sub>4</sub> as solvent confirmed the presence of an alcoholic OH; however, solvent absorption obscured the spectral region useful in assigning double bond isomerism. When CS<sub>2</sub> was used as solvent the IR of the compound showed a prominent band at 715 cm<sup>-1</sup> (*cis* double bond) but none in the 960-970 cm<sup>-1</sup> region (*trans* double bond). Collectively, the spectral information led to the conclusion that the melon component

<sup>1</sup> KEMP, T. R., STOLTZ, L. P. and KNAVEL, D. E. (1972) J. Agric. Food Chem. 20, 196.

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is cis,cis-3,6-nonadien-1-ol. An isomer of 3,6-nonadien-1-ol was synthesized by condensation of cis-4-heptenal with malonic acid in triethanolamine followed by decarboxylation, esterification with  $CH_2N_2$ , and reduction with  $LiAlH_4$ . The product obtained was trans,cis-3,6-nonadien-1-ol whose MS was very similar to that of the plant component. An IR spectrum revealed bands at 970 and 715 cm<sup>-1</sup> corresponding to trans and cis double bonds, respectively. As far as we could determine no isomer of 3,6-nonadien-1-ol has previously been reported in the literature.

After sampling of the plant component earlier, we stated that it had an odor which was musky or reminiscent of muskmelon.<sup>1</sup> However, upon a more complete investigation, it was found that the compound was unstable and that when tested shortly after GLC purification *cis,cis-3,6-nonadien-1-ol* was consistently described by a flavor panel as reminiscent of watermelon or watermelon rind. Watermelon was subsequently found to be a convenient source of the compound for flavor threshold estimation. The amount of compound obtained from fresh watermelon by vacuum steam distillation–extraction corresponded to a concentration of *ca* 200 ppb based on fresh melon weight. The flavor threshold concentration of *cis,cis-3,6-nonadien-1-ol* in aqueous solution was estimated at 10 ppb by the method of Patton and Josephson.<sup>2</sup>

Precursor	Aldehyde	Alcohol
9-Octadecenoic acid (oleic)	Nonanal	Nonan-1-ol
9.12-Octadecadienoic acid (linoleic)	trans-2-Nonenal	trans-2-Nonen-1-ol cis-3-Nonen-1-ol
9.15-Octadecadienoic acid (iso-linoleic)	cis-6-Nonenal	cis-6-Nonen-1-ol
9,12,15-Octadecatrienoic acid (linolenic)	trans, cis-2, 6-Nonadienal	trans, cis-2,6-Nonadien-1-ol cis, cis-3,6-Nonadien-1-ol

Table 1. C9 aldehydes and alcohols isolated from muskmelon essence shown with possible  $\rm C_{18}$  fatty acid precursors

Table 1 shows possible fatty acid precursors of cis,cis-3,6-nonadien-1-ol and other C<sub>9</sub> compounds identified in muskmelon essence. Included in Table 1 are *trans,cis-2,6*-nonadienal and *trans,cis-2,6*-nonadien-1-ol which have not been reported previously as muskmelon essence components. They were identified by MS and IR spectral data. With regard to biochemical origin, Grosch and Schwarz<sup>3</sup> have shown that the cucumber aldehydes, *trans-2*-nonenal and *trans,cis-2,6*-nonadienal are related to linoleic and linolenic acid, respectively. They postulated that *cis-3*-aldehydes are formed initially and that these rearrange to *trans-2*-aldehydes; however, the former compounds were not isolated. The six C<sub>9</sub> alcohols isolated from melon may have been formed by reduction of the corresponding aldehydes. The *cis-3*-alcohols should be more stable than the *cis-3*-aldehydes and for this reason more readily isolated from the fruit.

Initially all  $C_9$  aldehydes and alcohols were identified in an essence prepared by vacuum steam distillation-extraction of frozen muskmelon. As was shown earlier<sup>4</sup> the essence from frozen melon yielded 10-fold or greater amounts of these compounds than

<sup>&</sup>lt;sup>2</sup> PATTON, S. and JOSEPHSON, D. V. (1957) J. Food Res. 22, 316.

<sup>&</sup>lt;sup>3</sup> GROSCH, W. and SCHWARZ, J. M. (1971) Lipids 6, 351.

<sup>&</sup>lt;sup>4</sup> KEMP, T. R., KNAVEL, D. E. and STOLTZ, L. P. (1973) Phytochemistry 12, 2921.

the essence from fresh melon. In the case of frozen muskmelon the amount of *cis,cis*-3,6-nonadien-1-ol obtained by the steam distillation-extraction technique corresponded to a concentration of approximately 30 ppb based on frozen melon weight. The amount of *cis,cis*-3,6-nonadien-1-ol obtained from fresh muskmelon was not determined since the fresh fruit contained an interfering substance. However, it can be said that the concentration did not exceed 2 ppb. Values given for the concentration of watermelon and muskmelon constituents should be used for relative comparisons only, since their relationship to actual concentrations of compounds in the fruit is unknown. *trans,cis*-2,6-Nonadienal, as well as *trans*-2-nonen-1-ol identified earlier, were minor components in the frozen essence and were not present in the essence from fresh muskmelon in amounts sufficient to be detected by our methods.

Several naturally occurring C<sub>9</sub> aldehydes and alcohols in addition to *cis,cis*-3,6-nonadien-1-ol have flavors reminiscent of melon or cucumber. *cis*-6-Nonenal has a flavor reminiscent of melon or green melon and was isolated from muskmelon.<sup>5</sup> We have also identified this compound in watermelon essence (larger amount than in muskmelon) by MS and GLC retention data. Keppler *et al.*<sup>6</sup> and Parks *et al.*<sup>7</sup> reported that 6-nonenal was an off-flavor component of partially hydrogenated vegetable oils and dried milk, respectively. Forss *et al.*<sup>8</sup> concluded that *trans*-2-nonenal and especially *trans,cis*-2,6-nonadienal contribute to cucumber flavor. Earlier, Takei and Ono<sup>9</sup> attributed the flavor of cucumbers to 2,6-nonadienal and 2,6-nonadien-1-ol. Thus a group of closely related plant species appear to have evolved several structurally similar compounds as flavor components.

#### EXPERIMENTAL

NMR were recorded on a Varian HR100 instrument (with an internal field-frequency lock and equipped with a 1024-channel CAT) using CDCl<sub>3</sub> as solvent and TMS as internal standard; MS on an Hitachi RMU-6E double focusing instrument using an ionizing energy of 70 eV; and IR on a Beckman IR-8 equipped with a mirror beam condenser using  $CS_2$  as solvent.

Isolation. Muskmelons (Cucumis melo L. var. reticulatus Naud.) cv. Supermarket were grown on the University of Kentucky Experiment Station farm in Lexington and watermelons (Citrullus vulgaris Schrad) were purchased at a market. Rinds and seeds were removed and the flesh was macerated in a Waring blender. The macerate was then subjected to steam distillation-extraction in a water-recycling apparatus at  $60-70^{\circ}$  under red. pres. as described previously.<sup>1.10</sup> GLC analyses were carried out using 1.8 m × 6 mm o.d. stainless steel columns packed with (a) 20% SE30 and (b) 10% diethyleneglycol succinate (DEGS) coated on 60-80 mesh, acid washed, silanized Chromosorb W. Initial separation of melon essence was accomplished on SE30 using a program of 1°/min from 100°. The nonadienol was rechromatographed on DEGS operated isothermally at 80°. Purified cis,cis-3,6-nonadien-1-ol yielded the following major MS peaks (above m/e 40): m/e (%) 67(100), 93(69), 79(51), 41(46), 55(45), 81(31), 68(25), 77(24), 91(21), 53(19), 107(18), 80(17), 54(16), 96(16), 98(12) and M<sup>+</sup> 140(1) (C<sub>9</sub>H<sub>16</sub>O requires: 140·1200. Found: 140·1225). IR bands were observed at 3620, 3020, 2960, 1050 and 715 cm<sup>-1</sup>. NMR signals are listed in Results and Discussion. Solutions of cis,cis-3,6nonadien-1-ol were presented in a five-member panel for flavor threshold and quality evaluation.

Synthesis of trans, cis-3,6-nonadien-1-ol. Malonic acid (10.5 g) was added to cis-4-heptenal (12 g) and triethanolamine (12 g) and the whole allowed to stand at room temp. for 2 days, before being heated on a steam bath for 8 hr.<sup>11</sup> The liquid was acidifed (25% H<sub>2</sub>SO<sub>4</sub>), extracted with Et<sub>2</sub>O, and the solvent evaporated to give an oily residue. The residue was extracted with dil. Na<sub>2</sub>CO<sub>3</sub> and the alkaline soln acidified and

- <sup>5</sup> KEMP, T. R., KNAVEL, D. E. and STOLTZ, L. P. (1972) Phytochemistry 11, 3321.
- <sup>6</sup> KEPPLER, J. G., SCHOLS, J. A., FENNSTRA, W. H. and MEIJBOOM, P. W. (1965) J. Am. Oil Chemists' Soc. 42, 246.
- <sup>7</sup> PARKS, O. W., WONG, N. P., ALLEN, C. A. and SCHWARTZ, D. P. (1969) J. Dairy Sci. 52, 953.
- <sup>8</sup> FORSS, D. A., DUNSTONE, E. A., RAMSHAW, E. H. and STARK, W. (1962) J. Food Sci. 27, 90.
- <sup>9</sup> TAKEI, S. and ONO, M. (1939) J. Agric. Chem. Soc. Japan 15, 193.
- <sup>10</sup> KEMP, T. R., STOLTZ, L. P., SMITH, W. T. and CHAPLIN, C. E. (1968) Proc. Am. Soc. Hort. Sci. 93, 334.
- <sup>11</sup> LINSTEAD, R. P., NOBLE, E. G. and BOORMAN, E. J. (1933) J. Chem. Soc. 557.

extracted with Et<sub>2</sub>O as before. The Et<sub>2</sub>O soln was treated with CH<sub>2</sub>N<sub>2</sub> and then dried. This soln (50 ml) was added dropwise with stirring to LiAlH<sub>4</sub>(2 g) in Et<sub>2</sub>O (150 ml) cooled to  $-35^{\circ}$ .<sup>12</sup> The temp. was held at about  $-30^{\circ}$  throughout the addition and for 1 hr afterwards. EtOAc was added, then  $10^{\circ}_{\circ}$  H<sub>2</sub>SO<sub>4</sub>, and the organic layer passed through a sintered glass filter to remove the fine ppt. The *trans,cis*-3,6-nonadien-1-ol formed (0-8 g) was purified by GLC and yielded the following major MS peaks (above m/e 40):  $m/e(^{\circ}_{\circ})$  67(100), 93(36), 79(33), 41(28), 68(25), 55(24), 81(23), 77(18), 109(15), 91(14), 80(11), 96(10), 107(10), 54(9), 95(9) and M<sup>+</sup> 140(4). IR bands were observed at 3620, 3020, 2960, 1050, 970 and 715 cm<sup>-1</sup>. (C<sub>9</sub>H<sub>10</sub>O requires: 140-1200. Found: 140-160).

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<sup>12</sup> BROWN, W. G. (1951) Organic Reactions (ADAMS, R., ed.), Vol. 6, pp. 490-491, Wiley. New York.