amounts of geosmin in pond water and fish tissue should be feasible for other products. For example, geosmin was detected at the parts per billion level when samples of sugar beet molasses were examined by oil extraction. Geosmin is a known constituent of beet juice and sometimes causes a flavor problem with table beets (18). Geosmin also is sometimes the cause of a musty off-flavor in dry beans (19,20).

In conclusion, simple rapid methods are presented for concentrating and detecting geosmin in aqueous media and in fish tissue at the parts per billion level.

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Synthesis of Fatty 2-Oxazolines from Fatty Methyl 2,3-Epoxy Ester[1]

M.H. Ansari and **M. Ahmad***
Section of Oils and Fats, Department of Chemistry, Aligarh Muslim University, Aligarh-202001, India

Reaction of methyl *trans*-2,3-epoxyhexadecanoate (I) with benzonitrile in presence of boron trifluoride-etherate (BF_3-etherate) as catalyst has yielded *cis*-2-phenyl-4-tridecyl-5-carbomethoxy-2-oxazoline (II), methyl 2-hydroxy-3-benzamidohexadecanoate (IV) and methyl 2,3-dihydroxyhexadecanoate (III). On the other hand, reactions of I with acetonitrile and acrylonitrile have resulted in the formation of their corresponding hydroxyamides, methyl 2-hydroxy-3-acetamidohexadecanoate (VI) and methyl 2-hydroxy-3-acryloamidohexadecanoate (VII), respectively, along with the product (III) only. Pyrolysis of hydroxyamides (IV), (VI) and (VII) afforded their corresponding 2-oxazolines, *cis*-2-phenyl-4-tridecyl-5-carboxy-2-oxazoline (V), *cis*-2-methyl-4-tridecyl-5-carboxy-2-oxazoline (VIII) and *cis*-2-vinyl-4-tridecyl-5-carboxy-2-oxazoline (IX), respectively, in good yields. The products have been characterized with the help of spectral and microanalyses.

The interest in the biological and industrial potential (1) of 2-oxazolines has resulted in various synthetic procedures (1) for the introduction of five-membered nitrogen- and oxygen-containing heterocycles, i.e. 2-oxazoline, into a hydrocarbon chain. Studies of the reactions of various short chain epoxides with nitriles in presence of a catalyst leading to the formation of 2-oxazolines have been described (2-5). Smith et al. (6) have reported the preparation of 2-oxazolines in good yields under mild conditions from the reactions of various short-chain epoxides with acetonitrile and benzonitrile in presence of boron trifluoride-etherate (BF_3-etherate) as catalyst. The 2-oxazolines also have been prepared by the pyrolysis of hydroxyamino compounds (7). Scanning of the literature revealed that the reaction of nitriles with fatty 2,3-epoxy esters and pyrolysis of fatty hydroxyamido esters are not reported. These observations prompted us to carry out the conversion of fatty 2,3- epoxy and hydroxyamido esters into fatty 2-oxazolines using the above methods (6,7).

EXPERIMENTAL PROCEDURES

All melting points were taken on a Koflar hot plate

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*To whom correspondence should be addressed.

apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 621 spectrometer; ^1H NMR spectra were recorded on a Varian A-60 D spectrometer using tetramethylsilane (TMS) as internal standard, and chemical shifts were measured in ppm (δ) downfield from TMS. The abbreviations s, m, d, t and br stand for singlet, multiplet, dublet, triplet and broad, respectively. Mass spectra were obtained with a JEOL-JMS-D300 mass spectrometer at 70 eV. The figures in parentheses denote the postulated source of the ions along with intensity relative to the base peak.

Thin layer chromatographic (TLC) plates were coated with silica gel G (0.25 mm thickness), and a mixture of petroleum ether (bp, 60-80 C)-diethyl ether-acetic acid (60:40:1, v/v/v) was used as developing solvent. The spots were visualized after spraying with an aqueous solution (20%) of perchloric acid followed by heating at 120 C.

Methyl *trans*-2,3-epoxyhexadecanoate was prepared by metachloroperbenzoic acid oxidation of methyl ester of *trans*-2-hexadecenoic acid according to the procedure of Gunstone and Jacobsberg (8); *trans*-2-hexadecenoic acid was prepared from hexadecanoic acid by the method of Palameta and Prostenik (9), and methyl ester of *trans*-2-hexadecenoic acid was prepared by using a catalytic amount of sulfuric acid (1-2 drops) in absolute methanol. BF_3-etherate and nitriles were freshly distilled before use.

General procedure. A solution of equimolar amounts of methyl *trans*-2,3-epoxyhexadecanoate (I) (1 g, 3.5 mmol) and BF_3-etherate (0.5 ml, 3.5 mmol) as catalyst in appropriate nitrile (5-8 ml) as solvent and reagent was stirred at room temperature for 4-5 hr. The reaction mixture was poured into aqueous sodium hydrogen carbonate (5%), extracted with dry dichloromethane, washed with water and dried over anhydrous sodium sulfate. A viscous oil was obtained after evaporation of the solvent in vacuo.

Reaction of I with benzonitrile. The methyl ester (I) (1 g, 3.5 mmol) was treated with benzonitrile (5-8 ml) in the presence of BF_3-etherate (0.5 ml, 3.5 mmol) as described above. The crude viscous product obtained after usual work-up of the reaction mixture showed the presence of three components on TLC. It was chromatographed over a column of silica gel (20 g). Elution with petroleum ether-ether (95:5, v/v) gave II as a pale yellow solid which on crystallization from petroleum ether at 0 C gave yellow crystals (39%), m.p. 65 C (Found: C, 69.73; H, 11.43; N, 4.32. $C_{24}H_{27}NO_3$ requires: C, 69.68; H, 11,38; N, 4.27%); IR (KBr): 1730, 1645, 1600, 1520, 1450, 1080, 880 and 700 cm^{-1}; NMR (CCl_4):δ 0.90 (t, 3H), 1.27 (br, s, chain CH_2-), 4.41 (*m*, 1H), 4.96 (d, 10 Hz, 1H), 7.87 (m, 3H) and 7.97 (m, 2H); MS: m/z 387 (M^+, 0.1), 328 (M-$COOCH_3$, 7.4), 282 (M-C_6H_5CO, 3.1), 260 [204 + $(CH_2)_4$, 3.0], 246 [204 + $(CH_2)_3$, 1.0] 225 (328-C_6H_5CN, 3.2), 219 [204 + (CH_2+H), 3.0], 204 [M-$CH_3(CH_2)_{12}$, 8.5], 160 (219-$COOCH_3$), 146 (204-$COOCH_2$, 3.5), 122 (121 + H, 10.2), 121 ($C_6H_5CONH_2^+$, 8.5), 119 ($C_6H_5C{\overset{N}{\diagdown\!\diagup}}O^+$, 1.0), 105 ($C_6H_3CO^+$, 100), 77 ($C_6H_5^+$, 21.2) and 69 (146-$C_6H_5$, 12.5). Subsequent elution with petroleum ether-ether (90:10, v/v) afforded III as a white solid which on crystallization from petroleum ether (40-60 C) gave white crystals (8%), m.p. 55 C (Found: C, 69.60; H, 11.40; $C_{17}H_{34}O_4$ requires: C, 67.51; H, 11.33%); IR(KBr): 3430, 3280, 1725, 1460 and 1060 cm^{-1}; NMR ($CDCl_3$):δ 0.90 (t, 3H), 1.27 (br, s, chain CH_2-), 3.32 (br, m, 2H), 3.7 (m, 1H), 3.77 (s, 3H), 4.11 (d, 2.5 Hz, 1H); m/z 304 (M+2, 0.4), 303 (M+1, 1.8), 302 (M^+, absent), 284 (M-H_2O, 3.5), 266 (284-H_2O, 0.7), 243 (M-$COOCH_3$, 6.5), 225 (243-H_2O, 12.3), 213 [M-$CH(OH)COOH_3$, 7.64], 195 (213-H_2O, 1.2), 119 [M-$CH_3(CH_2)_{12}$, 12.8], 101 (119-H_2O, 2.0), 90 (McLafferty ion, 59.0), 87 (119-CH_3OH, 8.8), 83 (101-H_2O, 52.0), 60 (119-$COOCH_3$, 11.0) and 55 (100). Further elution with petroleum ether-diethyl ether (40:60, v/v) yielded IV as a white solid which on crystallization from dry dichloromethane gave white crystals (51%), m.p. 106 C (Found: C, 66.14, H, 11.42; N, 4.13. $C_{24}H_{39}NO_4$ requires: C, 66.06; H, 11.37; N, 4.05%); IR (KBr): 3370, 3270, 1730, 1630, 1600, 1520, 1450, 1070, 880 and 700 cm^{-1}; NMR (DMSO-d^6); δ 0.90 (t, 3H), 1.25 (br, s, chain CH_2-), 2.51 (br, s, 1H), 3.7 (s, 3H), 4.16 (br, m, 2H), 5.28 (d, 6Hz, 1H), 7.40 (m, 3H) and 7.78 (m, 2H); MS: m/z 405 (M^+, absent), 346 (M-$COOCH_3$, 10.8), 322 (M-C_6H_5, 1.0), 316 [M-$CH(OH)COOCH_3$, 4.9], 239 (316-C_6H_5, 2.0), 222 [M-$CH_3(CH_2)_{12}$, 0.6], 211 (316-C_6H_5CO, 12.5), 163 (222-$COOCH_3$, 2.0), 155 (163-H_2O, 5.0), 145 (222-C_6H_5, 3.5), 133 [222-$CH(OH)COOCH_3$, 5.5], 127 (145-H_2O, 7.5), 113 (145-CH_3OH, 10.0), 105 ($C_6H_5CO^+$, 20.0), 90 (McLafferty ion, 67.5) and 57 (100).

Reaction of I with acetonitrile. Reaction of I (1 g, 3.5 mmol) with acetonitrile (5-8 ml) finally yielded a crude viscous product which showed two components on TLC. It was chromatographed over a column of silica gel (20 g), and elution with petroleum ether-ether (90:10, v/v) gave pure product (III) (10.5%), m.p. 55 C. It had analytical and spectral data identical to that given earlier. Further elution with petroleum ether-ether (50:50, v/v) gave product (V) as a white solid which on crystallization from dichloromethane at room temperature gave white crystals (88%), m.p. 98 C (Found: C, 66.48; H, 10.9; N, 4.11. $C_{19}H_{37}NO_4$ requires: C, 66.39; H, 10.83; N, 4.07%); IR (KBr): 3370, 3270, 1717 and 1630 cm^{-1}; NMR ($CHCl_3$): δ 0.9 (t, 3H), 1.27 (br, s, chain CH_2-), 2.09 (s, 3H), 3.72 (m, 1H), 3.80 (s, 3H), 4.3 (br, m, 2H), 6.25 (d, 9Hz, 1H); MS: m/z 345 (M+2, 0.3), 344 (M+1, 2.0), 343 (M^+, 1.6), 312 (M-OCH_3, 1.5), 300 (M-CH_3CO, 0.6), 284 (M-$COOCH_3$, 11.2), 282 (306-H_2O, 26.2), 266 (284-H_2O, 12.5), 254 [M-$CH(OH)COOCH_3$, 99.4], 240 (254-CH_2, 28.1), 212 (254-$COCH_2$, 99.4), 160 (M-$CH_2)_{12}$, 3.7); 142 (160-H_2O, 10.0), 128 (160-CH_3OH, 3.7), 118 (160-$COCH_2$, 14.4), 101 (160-$COOCH_3$, 14.0), 90 (McLafferty ion, 4.4) and 43 (100).

Reaction of I with acrylonitrile. Similarly, treatment of I (1 g, 3.5 mmol) with acrylonitrile (5-8 ml) yielded a crude product which showed two components on TLC. The crude product was chromatographed over a column of silica gel (20 g). Elution with petroleum ether-ether (89:11, v/v) gave pure III (11%), m.p. 55 C, showing the same analytical and spectral data as given earlier. Subsequent elution with petroleum ether-ether (45:55, v/v) afforded the product (VI) as a white solid which on crystallization from dichloromethane at room temperature gave white crystals (85%), m.p. 48 C (Found: C, 67.61, H, 11.1; N, 4.52. $C_{20}H_{37}NO_4$ requires: C, 67.56; H, 10.49; N, 4.41%); IR (KBr): 3355, 3265, 1720, 1650 and 1620 cm^{-1}; NMR ($CDCl_3$): δ 0.90 (t, 3H), 1.27 (br, s, chain CH_2-), 2.24 (d, 7Hz, 1H), 2.5 (m, 1H), 3.79 (s, 3H), 4.2

(m, 2H), 6.15 (t, 4Hz, 1H), 7.0 (d, 3Hz, 2H); MS: m/z 357 (M+2, 0.1), 356 (M+1, 1.4), 355 M^+, absent), 3.28 (M-$CH{=}CH_2$, 0.1), 3.24 (M-OCH_3, 0.2), 296 (M-$COOCH_3$, 4.1), 278 (296-H_2O, 1.4), 267 (266+H, 20.6), 266 [M-CH(OH)$COOCH_3$, 100], 240 (266-CH=CH, 5.4), 212 (266-CH=CH.CO, 88.8), 184 (172+CH_2, 2.5), 172 [M-$CH_3(CH_2)_{12}$, 2.5], 154 (172-H_2O, 1.2), 140 (172-CH_3OH, 2.5), 118 (172-CH=CH.CO, 2.5), 114 (172-$COOCH_2$, 3.7), 90 (McLafferty ion, 2.5), 55 (CH_2=CH.CO^+, 85.0) and 43 (25.0).

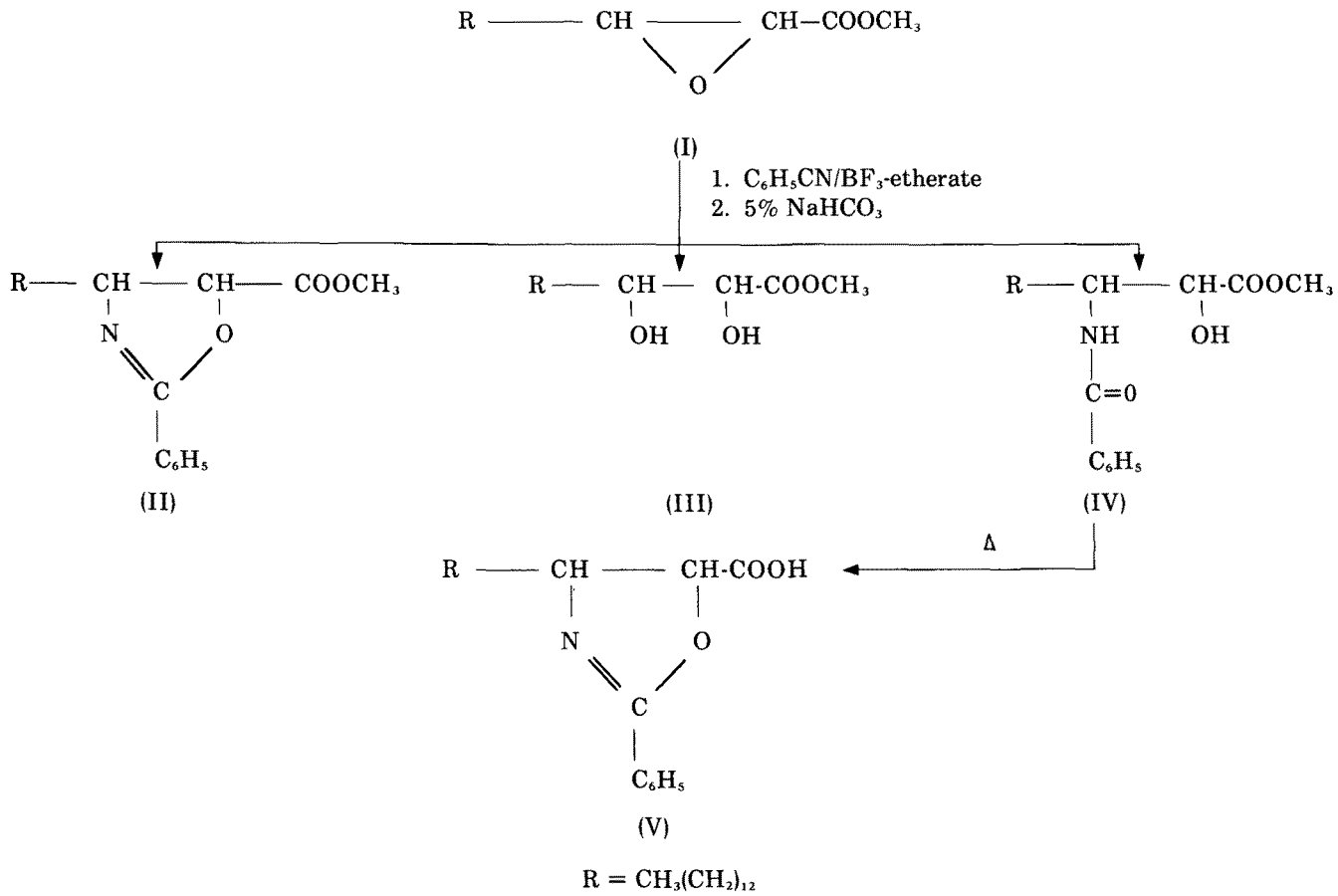
Pyrolysis of hydroxyamide (IV). The hydroxyamide (IV) (1.5 mmol) was heated at 210-220 C for 6-8 hr under the atmosphere of nitrogen. The cooled pyrolysate was dissolved in ether, filtered and dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo to give a viscous oil. It was chromatographed over a column of silica gel (10 g). Elution with a mixture of petroleum ether-ether (92:8, v/v) gave product (V) as a pale yellow solid (75.5%), m.p. 81 C. IR (KBr): 3370, 1700, 1645, 1600, 1525, 1450, 1070, 880 and 705 cm^{-1}; NMR (CCl_4): δ 0.90 (t, 3H), 1.28 (br, s, 24H), 4.4 (m, 1H), 4.9 (d, J=10Hz, 1H), 7.85 (m, 3H, aromatic), 7.96 (m, 2H, aromatic) and 9.1 (br, s, 1H).

Pyrolysis of hydroxyamide (VI). Similarly, pyrolysis of VI (1.5 mmol) as described above finally afforded a brown viscous oil which was chromatographed over a column of silica gel (10 g). Elution with a mixture of petroleum ether-ether (95:5, v/v) gave product (VIII) as a colorless oil (70.2%). IR (neat): 3350, 1705, 1650, 1530, 1450, 1370, 1210, 1080 and 720 cm^{-1}; NMR (CCL_4): δ 0.87 (t, 3H), 1.27 (br, s, chain CH_2-), 1.9 (s, 3H), 3.75 (m, 1H), 4.9 (d, 10 Hz, 1H) and 9.1 (br, s, 1H); MS: m/z 312 (M+1, 0.2), 311 (M^+, 0.3), 296 (M-CH_3, 1.0), 270 (M-CH_3CN, 1.0), 268 [M-CH_3CO or $CH_3(CH_2)_2$, 3.0], 2.66 (M-COOH, 1.5), 254 [M-CH_3CON or $CH_3(CH_2)_3$, 16.0], 240 (254-CH_2, 2.4), 237 [M-CH(O)COOH or 254-OH, 1.0], 236 (254-H_2O, 1.0), 225 (270-COOH, 4.2), 212 (128+$(CH_2)_6$, 25.5), 185 (128+$(C_4H_8$+H), 5.6), 183 (M-128, 2.5), 171 (185-CH_2, 4.1), 157 (171-CH_2, 3.7), 143 [128+$(CH_2$+H), 4.4], 129 (128+H, 16.0), 128 [M-$CH_3(CH_2)_{12}$, 3.7)], 125 (143-H_2O, 8.1), 113 (128-CH_3, 11.6), 112 (129-OH, 17.8), 111 (129-H_2O, 14.4), 110 (128-H_2O, 7.2), 87 (128-CH_3CN, 11.0), 83 (128-COOH, 31.2), 57 (100) and 43 (97.5).

Pyrolysis of hydroxyamide (VII). Pyrolysis of VII (1.5 mmol) as described earlier also yielded a brown viscous oil. It was chromatographed over a column of silica gel (10 g). Elution with a mixture of petroleum ether-ether (94:6, v/v) gave product (IX) as a colorless oil (69%). IR (neat): 3380, 1700, 1655, 1620, 1525, 1445, 1360, 1220, 1060 and 715 cm^{-1}; NMR (CCl_4): δ 0.89 (t, 3H), 1.27 (br, s, chain CH_2-), 4.25 (m, 1H), 4.92 (d, 10Hz, 1H), 6.14 (t, 1H) and 6.78 (d, 9Hz, 2H).

RESULTS AND DISCUSSION

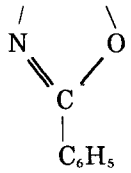
Methyl *trans*-2,3-epoxyhexadecanoate (I) on treatment with benzonitrile in presence of BF_3-etherate (Scheme 1)



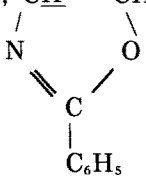
Scheme 1.

resulted in the formation of three products (II, III, IV). The product (II) was analyzed correctly for $C_{24}H_{37}NO_3$. It revealed characteristic IR bands at 1730 ($\underline{COO}CH_3$) and 1645 for C=N stratching frequency of oxazoline ring. The bands at 1600, 1450 and 880 cm^{-1} accounted for the presence of benzene ring. The NMR spectrum of this compound showed characteristic signals at δ 7.97 (2H, aromatic), 7.87 (3H, aromatic),

4.96 d (1H, J=10Hz, -CH——C$\underline{H}$-COOCH$_3$),



4.41 m (1H, -C$\underline{H}$——CH-COOCH$_3$),



3.75 (3H, -COOC$\underline{H}_3$), 1.27 br, s (chain-C$\underline{H}_2$ protons) and 0.9 t (3H, terminal-C$\underline{H}_3$). From the coupling constants (3) of 4-methine and 5-methine protons, the configuration of these two protons in oxazoline ring was established, and it was confirmed that they were in *cis*-position. These data confirmed the structure of the product (II) as *cis*-2-phenyl-4-tridecyl-5-carbomethoxy-2-oxazoline. The MS fragments of II at m/z 387 (M^+), 328, 204, 146 and 105 (base peak) further supported the proposed structure.

The compound (III) was analyzed for $C_{17}H_{34}O_4$. It showed IR bands at 3430 and 3280 for hydroxyl groups and 1725 cm^{-1} for ester carbonyl group. The NMR spectrum gave characteristic signals at δ 3.32 br, m (2H, 2 × O$\underline{H}$, D_2O exchangeable),

3.7 m (1H, -C$\underline{H}$-CH-COOCH$_3$,
OH OH

in part merged with ester methyl signal) and

4.11 d (1H, J=2.5 Hz,-C$\underline{H}$-COOCH$_3$)
OH

along with other usual signals of fatty acid methyl ester. These data established the structure of III as methyl 2,3-dihydroxyhexadecanoate. The mass spectrum further supported the above structure. It gave no molecular ion peak at m/z 302 but gave other structure revealing ion peaks at m/z 304, 303, 243, 225, 213, 119 and 90.

The product (IV) was found to have the composition, $C_{24}H_{39}NO_4$. Its IR spectrum showed characteristic bands at 3370 (OH), 3270 (N-H), 1730 ($\underline{COO}CH_3$) cm^{-1} (NH$\underline{CO}$). The bands at 1600, 1450, 880 cm^{-1} accounted for the presence of benzene ring. The NMR spectrum gave characteristic signals at δ 2.51 m (1H, -O$\underline{H}$, D_2O exchangeable),

4.16 m (2H, -C$\underline{H}$——C$\underline{H}$-COOCH$_3$),
-NH OH

5.28 d (1H, J=6Hz, -N-$\underline{H}$), 7.4 m (3H, aromatic) and 7.78 m (2H, aromatic) along with the other usual signals of fatty acid methyl ester. The structure of the product (IV) was further confirmed by its mass spectral data. The positions of the hydroxyl group at C_2 and benzamido group at C_3 were confirmed by the ion peaks at m/z 316, 222 and 90. On the basis of the above data, IV was characterized as methyl 2-hydroxy-3-benzamidohexadecanoate.

Reaction of I with acetonitrile (Scheme 2) afforded the product (VI) along with III. The product (VI) was found to have a molecular formula $C_{19}H_{37}NO_4$. Its IR spectrum showed absorption bands at 3370 (OH), 3270 (N-H), 1717 ($\underline{COO}CH_3$) and 1630 cm^{-1} (NH$\underline{CO}$). The NMR spectrum of VI exhibited characteristic bands at δ 2.09 s (3H, -NHCOC$\underline{H}_3$), 3.72 m (1H, -CH-O$\underline{H}$, D_2O exchangeable, in part merged with ester methyl signal),

4.3 br, m (2H, -C$\underline{H}$-C$\underline{H}$-COOCH$_3$),
-NH OH

6.25 d (1h, J=9Hz, -N$\underline{H}$, slow D_2O exchangeable) along with other usual signals of fatty acid methyl ester. The structure of the product (VI) was further confirmed by its mass spectral data. It gave molecular ion peak 343 (M^+) along with M+1 and M+2 peaks at 344 and 345, respectively. The ion peaks at m/z 254, 160 and 90 confirmed the position of hydroxyl group at C_2 and acetamido group at C_3 in the chain. These data confirmed the structure of the product (VI) as methyl 2-hydroxy-3-acetamidohexadecanoate.

Reaction of I with acrylonitrile (Scheme 2) gave product (VII) along with III. The product (VII) gave elemental analysis corresponding to the molecular formula $C_{20}H_{37}NO_4$. Its IR spectrum revealed absorption bands at 3355 (OH), 3265 (N), 1720 ($\underline{COO}CH_3$), 1650 (-NH$\underline{CO}$) and 1620 cm^{-1} (C=C). The NMR spectrum gave characteristic signals at δ 2.24 d (1H, J=7Hz, -N$\underline{H}$), 2.5 m (1H, -O$\underline{H}$, D_2O exchangeable),

4.2 br, m (2H, -C$\underline{H}$-C$\underline{H}$-COOCH$_3$),
-NH OH

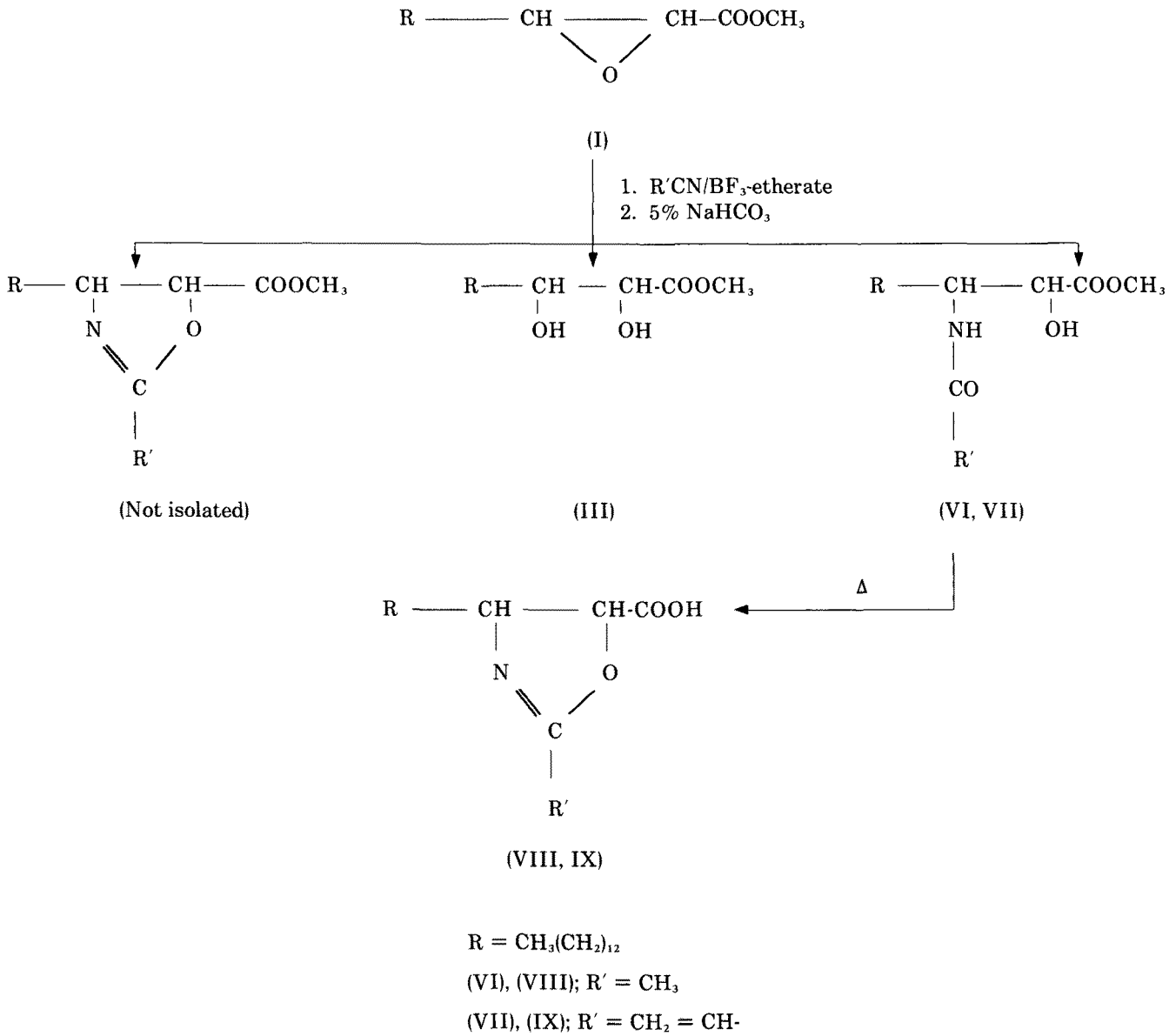
6.15 t (1H, J=4Hz, CH_2=C$\underline{H}$-CO-) and 7.0 d (2H, J=3Hz, C$\underline{H}_2$=CH-CO-) along with other usual signals of fatty acid methyl esters. The structure of the product (VII) was confirmed by its mass spectral data. It gave no molecular ion peak at m/z 355 but gave other salient ion peaks at m/z 356 (M+1), 296, 266, 212 and 90. These data confirmed the structure of the product (VII) as methyl 2-hydroxy-3-acrylamidohexadecanoate.

Methyl 2-hydroxy-3-benzamidohexadecanoate (VI), when heated at 210-220 C for 6-8 hr under the atmosphere of nitrogen, yielded a dark brown viscous pyrolysate which on quick silica gel column chromatographic fractionation gave a pale yellow product (V),

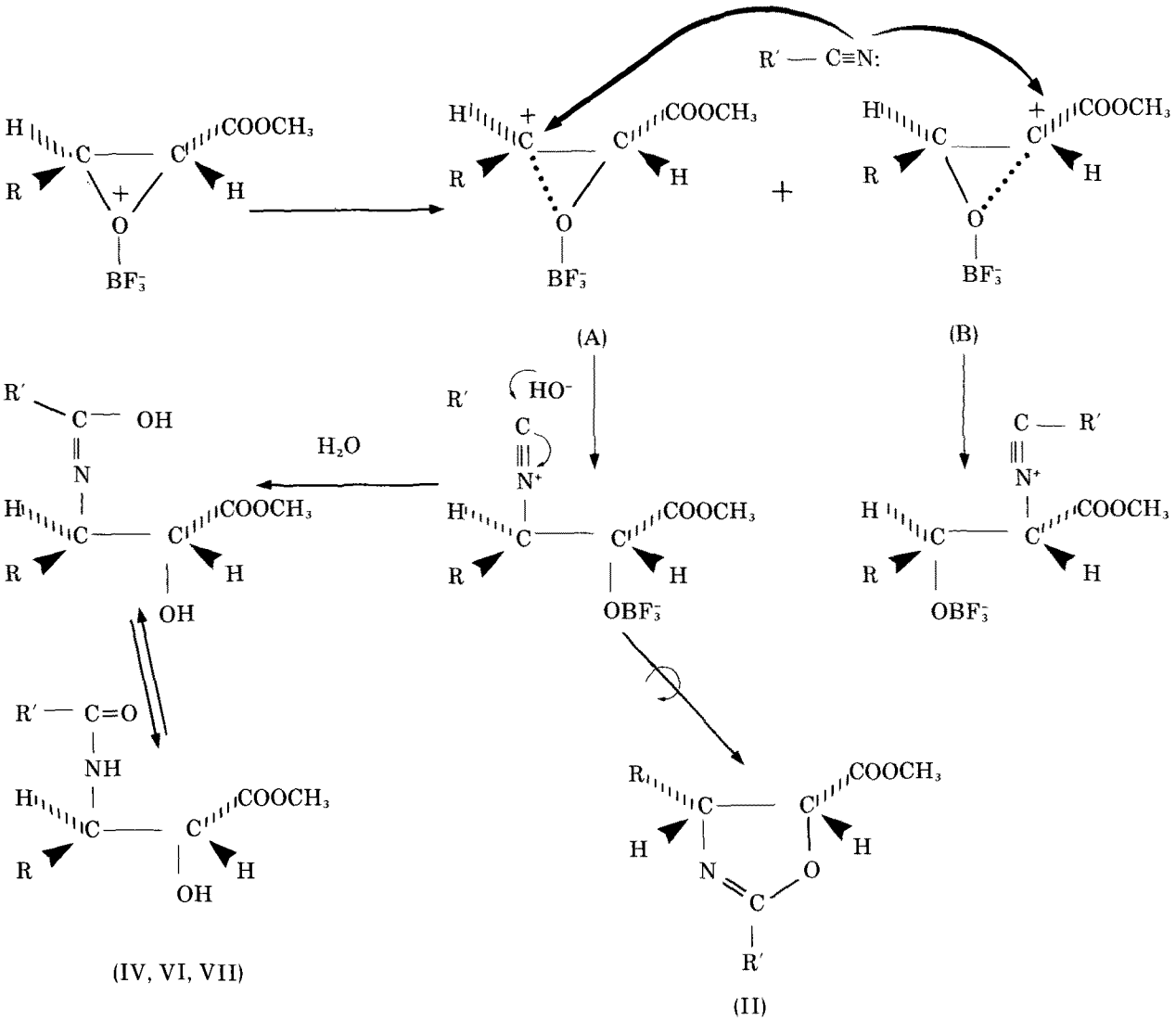
m.p. 81 C (Scheme 1). The IR spectrum of the product (V) instead of ester carbonyl absorption band at ~1730 cm^{-1} showed bands at 3370 (COOH) and 1700 cm^{-1} (COOH) which proved that the ester group of hydroxyamide (V) was changed into carboxyl group. A characteristic band at 1645 cm^{-1} was attributed to the presence of the C=N group. The NMR spectrum of V was found similar to that of II except for the presence of carboxylic acid proton signal at δ 9.1 instead of ester methyl protons signal. These data clearly established the structure of V as *cis*-2-phenyl-4-tridecyl-5-carboxy-2-oxazoline.

Pyrolysis of methyl 2-hydroxy-3-acetamidohexadecanoate (VI) as described above on silica gel column chromatographic fractionation afforded a colorless product (VIII) as a liquid (Scheme 2). The IR spectrum of the product (VIII) instead of ester carbonyl absorption band ~1730 cm^{-1} exhibited bands at 3350 and 1705 cm^{-1} for carboxyl group. It also gave a band at 1650 cm^{-1} which was attributed to the C=N stretching of the oxazoline ring. The NMR spectrum gave characteristic signals at δ 1.9 s (3H, methyl proton of ring), 3.75 m (1H, 4-methine proton of ring), 4.9 d (1H, J=10Hz, 5-methine proton of ring) and 9.1 br, s (1H, COOCH) along with other usual signals of fatty acid chain. These data confirmed the structure of VIII as *cis*-2-methyl-4-tridecyl-5-carboxy-2-oxazoline. The MS fragments at m/z 311 (M^+) , 266, 254, 129, 128 and 83 further supported the above structure.

Similarly, pyrolysis of methyl 2-hydroxy-3-acrylamidohexadecanoate (VII) as described earlier gave product (IX) as a colorless liquid (Scheme 2). The



Scheme 2.



Scheme 3.

product (IX) in its IR spectrum showed bands at 3380 and 1700 (COOH), 1655 (C=N) and 1620 cm^{-1} (C=C). The NMR spectrum gave characteristic signals at δ 4.25 m (1H, 4-methine proton of ring), 4.92 d (1H, J=10Hz, 5-methine proton of ring), 6.14 t (1H, CH_2=C<u>H</u>-), 6.78 d (2H, J=9Hz, C<u>H</u>$_2$=CH-) and 9.1 br, s (1H, -C<u>O</u>O<u>H</u>) along with other usual signals of fatty acid chain. On the basis of the above data, IX was characterized as *cis*-2-vinyl-4-tridecyl-5-carboxy-2-oxazoline.

The opening of *trans*-2,3-epoxy acid ester (I) was found to proceed stereospecifically with inversion in configuration to give *cis*-2-oxazoline (II). The preferred formation of only 4-tridecyl-5-carbomethoxy-*cis*-2-oxazoline (II) and methyl 2-hydroxy-3-amido esters (IV, VI and VII) can be explained on the basis of the mechanism (Scheme 3) in which the nitrile moiety preferably attacks at complex (A) and not complex (B). It may be due to the negative inductive effect caused by the adjacent electron-withdrawing carbonyl group in complex (B).

Non-isolation of the corresponding 2-oxazolines, although formed in low yield as revealed by TLC, from the reaction of I with aceto- and acrylonitrile, may be due to their low stability and prompt hydrolysis to the corresponding hydroxyamides (VI) and (VII) during work-up. *cis*-2-Oxazoline (II), although formed in low yield in comparison to the yield reported in the literature (6), was isolated. It may be due to its greater stability, because it has been reported (7) that the 2-oxazolines having aryl substitution at the C_2 position in the ring have shown more stability than those having alkyl substitution. These products (II, V, VIII and IX) after three days were found contaminated with their hydroxyamides; after seven days they were completely hydrolyzed.

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A High Performance Size-Exclusion Chromatographic Method for Evaluating Heated Oils[1]

Pamela J. White* and **Yen-chin Wang**
Food and Nutrition Department, Iowa State University, Ames, IA 50011

High performance size-exclusion chromatography (HPSEC) was used to measure compounds with high-molecular weight (MW) formed during the heating of oil. Formation of the high-MW compounds is believed to be a reliable indicator of heat abuse in oils. The HPSEC method employs two μ-spherogel size-exclusion columns (500 and 1000 Å) in a series to separate the high-MW compounds which are detected at a wavelength of 234 nm by using a variable wavelength detector.

The method was examined in the following study. Two sources of soybean oil were heated under laboratory conditions at 182 ± 2 C for eight 7-hr days. Samples were taken periodically and tested by using HPSEC. Oil samples from two commercial deep-fat frying operations were similarly tested. In all cases, size, number and apparent MW of the compounds formed increased with increasing frying time.

The HPSEC procedure was compared with a method involving separation of polar and nonpolar components in a used frying fat by means of column chromatography on silica gel.

During deep-fat frying, volatile and nonvolatile decomposition products are formed from the oil. The volatile ones distill out during frying, but the nonvolatile products accumulate in the oil and form higher and higher molecular weight (MW) compounds as heating progresses. This changes the functional, nutritional and sensory properties of the fat or oil, resulting in a darkened color, an increased viscosity, a decreased smokepoint and increased foaming of the oil (1-4). This, in turn, is said to cause an increased absorption of oil into the food being fried and an overall decrease in food quality. The high-MW compounds also have been suggested by some researchers to be harmful when fed at high levels to rats (5-10).

The measurement of heat abuse in oils has proved to be a challenge. It is difficult to volatilize the high-MW compounds for gas chromatography purposes and to dissolve the abused oils in solvents for liquid chromatography. Traditionally, overall values such as free fatty acid (11,12), iodine value (13,14), nonurea-adduct forming esters (15,16) and viscosity (12,15) have been used to assess heat damage in frying oils. However, none of these methods has proved to be a good measure of heat abuse.

A column chromatography (CC) method that measures the polar materials in an oil does give a good indication of total oil abuse, but it is not specific (17). A quick method that measures the dielectric constant has been used to estimate frying oil degradation (18). Again, the dielectric constant method gives only one overall value. It would be difficult to adapt the procedure to real situations because the reading is influenced by many outside factors, such as water or fat extracted from the fried food (19-21). In addition, Graziano (18) reported that fresh oils differ in dielectric constants, so the instrument must be standardized each time an oil is tested. In the last 10 years, researchers have developed methods for measuring the high-MW fraction or portions of it. The high-MW compounds should be a more reliable indicator of heat abuse because of their low volatility and resultant increased stability (20,21).

Aitzetmüller published a series of articles about using liquid chromatography (LC) to estimate the artifacts in heated oils (22-24). Each was an improvement upon the previous method. He was able to measure the total polar artifacts by gradient elution and a moving wire detector (24). In 1978, however, Billek et al. (25) compared the LC method with several others and found Aitzetmüller's method to have poor reproducibility and to be very time consuming.

Several researchers have used gel permeation chromatography to study the formation of high-MW compounds in heated oil (26-28). The major drawbacks to these methods have been incomplete and lengthy (5-6 hr) separations.

Firestone (29) used gas liquid chromatography (GLC) to determine differences between oxidative and thermal dimers in heated oils. The dimers have been shown to

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*To whom correspondence should be addressed.