the two phase system was better than that of the emulsion system.

The results indicate that the present method is a very simple and rapid one to determine free fatty acids, and is suitable for the determination of lipase activity.

REFERENCES

1. Brockman, H. L., in *Lipases*, edited by B. Borgström and H. L. Brockman, Elsevier Sci. Pub., Amsterdam, 1984, pp. 3-46.
2. Linfield, W.M., R.A. Barauskas, L. Sivieri, S. Serota and R.W. Stevensor Sr., *JAOCS 61*:191 (1984).
3. Macrae, A.R., *JAOCS 60*:291 (1983).
4. Kwon, D.Y., and J.S. Rhee, *Korean J. Chem. Eng. 1*:153 (1984).
5. Blain, J.A., M.W. Akhtar and J.D.E. Patterson, *Pak. J. Biochem. 10*:41 (1976).
6. Kim, K.H., D.Y. Kwon and J.S. Rhee, *Lipids 19*:975 (1984).
7. Jensen, R.G., *Lipids 18*:650 (1983).
8. Duncombe, W.G., *Biochem. J. 88*:7 (1963).
9. Hron, W.T., and L.A. Menahan, *J. Lipid Res. 22*:377 (1981).
10. Sahasrabudhe, M.R., *JAOCS 59*:354 (1982).
11. Bowyer, D.E., J.S. Cridland and J.P.King, *J. Lipid Res. 19*:274 (1978).
12. Radding, W., G.G. Mayer and J.W. Correll, *J. Lipid Res. 24*:100 (1983).
13. Shipe, W.F., G.F. Senyk and K.B. Fountain, *J. Dairy Sci. 63*:193 (1980).
14. Bains, G.S., S.V. Rao and D.S. Bhatia, *JAOCS 41*:831 (1964).
15. Lowry, R.R., and I.J. Tinsley, *JAOCS 53*:470 (1976).
16. Leuenberger, H.G.W., in *Biotechnology*, edited by H.-J. Rehm and G. Reed, Verlag Chemie, Weinheim, 1984, Vol. 6a pp. 5-29.
17. Riddick, J.A., and W.B. Bunger, in *Organic Solvents*, edited by A. Weissberger, 3rd edn., John Wiley & Sons, New York, 1970, pp. 95-108.
18. Singleton, W.S., in *Fatty Acids*, edited by K.S. Markley, Interscience Pub. Inc., New York 1960, pp. 609-678.
19. Kwon, D.Y., and J.S. Rhee, *Korean J. Food Sci. Technol. 17*:490 (1985).

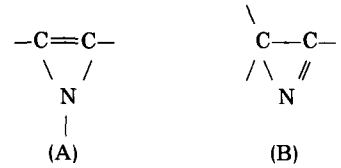
[Received June 28, 1985]

Addition of Phthalimidonitrene to Acetylenic Fatty Acid Esters: Synthesis of Long-Chain 2-Phthalimido-2H-Azirines

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

Lead tetraacetate (LTA) oxidations of N-aminophthalimide in the presence of acetylenic fatty acid esters have resulted in the formation of corresponding 1H-azirines that spontaneously rearranged to give 2H-azirines in moderate yields. 2H-Azirine derivatives (IV, V and VI) of acetylenic fatty acid esters, methyl 10-undecynoate (I), methyl 9-undecynoate (II) and methyl 9-octadecynoate (III), respectively, have been prepared and characterized with the help of spectral and micro analyses.

To continue our studies on the synthesis of long chain N-aminoaziridines (12) by the addition of aminonitrene intermediate to olefins, we focused on monounsaturated analogs of aziridine, i.e., 1H-azirine (A) and 2H-azirine (B).

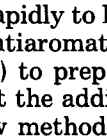


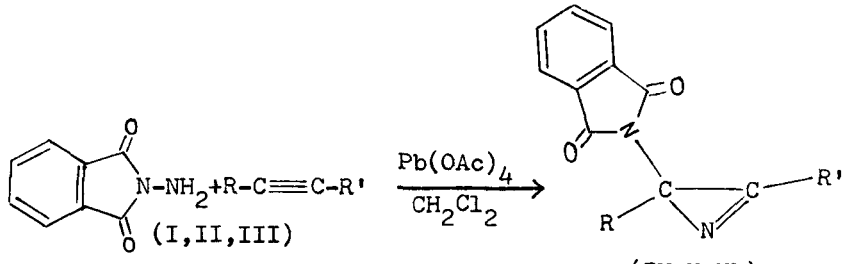
(A) (B)

No azirine (A) has been isolated yet or even demonstrated clearly to be a reaction intermediate. However, it is believed that A is formed first and then rearranges very rapidly to B. The rearrangement may be due to the high antiaromatic (3,4) nature of A. A number of methods (5-8) to prepare azirines are described in the literature, but the addition of nitrene to acetylenes is a relatively new method that gave a fairly good yield of azirine in one step. Anderson et al. (9) first described the addition of aminonitrene to acetylenes and reported the formation of B, which probably occurred by the rearrangement of A. We report here the synthesis of chain-substituted 2H-fatty azirine by the reaction of acetylenic fatty acid esters (terminal, penultimate, internal) with the nitrene intermediate generated in situ by the LTA oxidation of N-aminophthalimide.

*To whom correspondence should be addressed.

RESULTS AND DISCUSSION

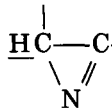
The oxidation of N-aminophthalimide in the presence of methyl 10-undecynoate (I) (Scheme 1), using LTA as an oxidant at room temperature on final work up and column chromatographic fractionation, gave an inseparable isomeric B (IV). Its infrared (IR) spectrum showed a characteristic sharp band at 1775 cm^{-1}, which has been assigned to the highly strained carbon nitrogen double bond vibration of the azirine ring (10). A broad band in the region of 1740-1680 revealed the presence of carbonyl functions of ester and phthalimido groups. Bands at 1600 and 1455 cm^{-1} accounted for C=C stretching of the benzene ring, a band at 1070 cm^{-1} accounted for C—H bending and one at 705 cm^{-1} accounted for an out-of-plane ring by sextants of the benzene ring. Its NMR spectrum gave a sharp multiplet at δ8.57 showing long range coupling () and a multiplet at δ7.82 for four protons of the benzene ring along with usual signals of fatty methyl ester. These data confirmed the structure of product IV as 2-(8-carbomethoxyoctyl)-2-phthalimido-2H-



Where:
I, R = H; R' = $(CH_2)_8COOCH_3$
IV, When R = H; R' = $-(CH_2)_8COOCH_3$ and R = $-(CH_2)_8COOCH_3$; R' = H
II, R = CH_3; R' = $-(CH_2)_7COOCH_3$
V, When R = CH_3; R' = $-(CH_2)_7COOCH_3$ and R = $-(CH_2)_7COOCH_3$; R' = CH_3
III, R = $CH_3(CH_2)_7-$; R' = $-(CH_2)_7COOCH_3$
VI, When R = $CH_3(CH_2)_7-$; R' = $-(CH_2)_7COOCH_3$ and R = $-(CH_2)_7COOCH_3$; R' = $CH_3(CH_2)_7-$

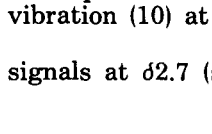
SCHEME 1

azirine. Appearance of a weak multiplet at δ4.65 for

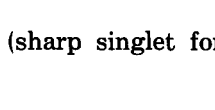


3-(8-carbomethoxyoctyl)-2-phthalmido-2H-azirine, in a small amount. The structure of azirine (IV) was confirmed by mass spectral data. The prominent mass ion peaks were observed at m/e 356 (M^+, 27), 201 (66, β-cleavage with two hydrogen transfers to the azirine ring) 187 (63, α-cleavage with two hydrogen transfers to the azirine ring) and 147 (53, C—N bond cleavage with one hydrogen transfer toward the phthalimido ring side). The other structure-revealing ion peaks observed are given in the Experimental section.

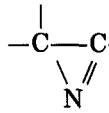
Similar oxidation with methyl 9-undecynoate (II) (Scheme 1) afforded azirine (V) that also was obtained as an isomeric mixture of inseparable positional isomers. The V IR spectrum also gave characteristic C=N azirine ring vibration (10) at 1774 cm^{-1}. The appearance of NMR signals at δ2.7 (sharp singlet for



(sharp singlet for

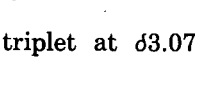


—, along with usual signals of fatty acid



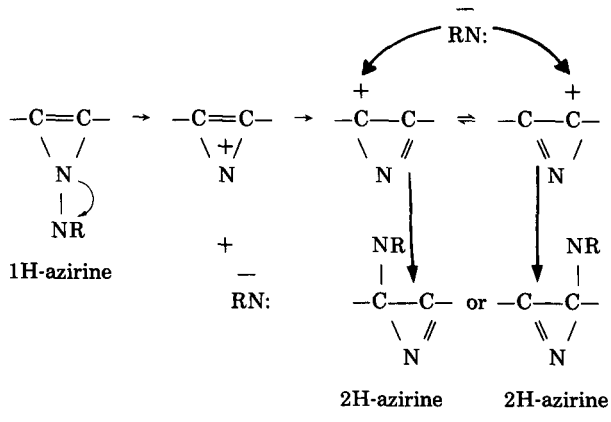
methyl ester, established the formation of the isomeric azirines as 2-(7-carbomethoxyheptyl)3-methyl-2-phthalimido-2H-azirine and 3-(7-carbomethoxyheptyl)2-methyl-2-phthalimido-2H-azirine. From methyl proton signals, the percentages of two isomers are calculated as 52.9% and 47.1% for the former and latter isomers, respectively. Mass spectrum exhibited ion peaks at m/e 356 (M^+, 3), 213 (6, β-cleavage to azirine ring), 201 (25, α-cleavage with two hydrogen transfers) and 199 (8, α-cleavage to azirine ring) and 147 (45, C—N bond cleavage with one hydrogen transfer toward the phthalimido ring side). Other structure-revealing ion peaks are given in the Experimental section.

Similarly, oxidation of methyl 9-octadecynoate (III) gave azirine (VI), which was assumed to be a mixture of nonseparable positional isomers and characterized as 2(3)-(7-carbomethoxyheptyl)-3(2)-octyl-2-phthalimido-2H-azirine. The IR spectrum of azirine (VI) showed a characteristic absorption band at 1775 cm^{-1} for C=N stretching in the azirine ring (10). The NMR spectrum gave a triplet at δ3.07 for



, and a multiplet centered at δ7.70 for four protons of the benzene ring, along with usual signals of fatty methyl ester. The structure was supported further by mass spectral data. It gave structure-revealing ion peaks at m/e 454 (M^+, 1), two β-cleavages to the azirine ring at 355 (2) and 311 (3) and two α-cleavages toward both the side of ring at 341 (3) and 297 (4), 147 (13, C—N bond cleavage toward the phthalimido ring side with one hydrogen transfer). Other structure-revealing ion peaks are given in the Experimental section.

The A to B rearrangement should give rise to isomeric azirines. In case of terminal acetylene (I), one isomer [2-(8-carbomethoxyoctyl)-2-phthalimido-2H-azirine] predominates over the other [3-(8-carbomethoxyoctyl)-2-phthalimido-2H-azirine], while in penultimate and internal acetylenes, a 1:1 mixture of both isomers is expected as observed in the case of penultimate acetylene (II). This behavior can be explained on the basis of the mechanism proposed by Anderson et al. (9) as shown:



In the case of I, two structural carbonium ions are possible, tertiary and secondary. Addition of phthalimido anion to the more stable carbonium ion results in the formation of azirine, 2-(8-carbomethoxyoctyl)-2-phthalimido-2H-azirine, in major amount; in penultimate (II) and internal (III) acetylenes, the formation of two structural carbonium ions of almost equal stability results in approximately a 1:1 ratio of both isomers.

EXPERIMENTAL PROCEDURES

All melting points are uncorrected and were taken on Kofler hot plate apparatus. IR spectra were recorded on a Perkin Elmer 621 spectrometer. Nuclear magnetic resonance spectra were obtained in 1% $CCl_4/CDCl_3$ on a Varian A-60 spectrometer using TMS as internal standard, and chemical shifts were measured in ppm (δ) downfield from TMS. The abbreviations s, m, d, t and br were used as singlet, multiplet, doublet, triplet and broad, respectively. Mass spectra were run on a JEOL JMS-D 300 mass spectrometer.

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

MATERIALS AND METHODS

10-Undecenoic and *cis*-9-octadecenoic acids were obtained commercially. 10-Undecynoic (mp 42 C, lit. [11], mp 41–43 C) and 9-octadecynoic (mp 45 C, lit. [11], mp 44–45 C) acids were prepared by bromination-dehydrobromination of respective olefinic acids using alcoholic potassium hydroxide by the method of Kannan et al. (11). Ames and Bowman's method (12) was used for the preparation of 9-undecynoic (mp 59 C, lit. [12], mp 58–59 C) acid, involving bromination of 10-undecenoic acid and dehydrobromination at 180 C using aqueous potassium hydroxide.

Methyl esters of the corresponding acids were prepared by using a catalytic amount of sulphuric acid in absolute methanol. N-aminophthalimide was prepared from phthalimide using the method of Drew and Hatt (13).

General procedure. Methyl ester of acetylenic fatty acids (3 mmol) was stirred with N-aminophthalimide (0.6 mmol) in dry dichloromethane for 20–25 min followed by addition of LTA (0.66 mmol) in portions over 15 min. The reaction mixture was stirred for an additional 20 min, filtered and washed with dichloromethane. The filtrate was washed with water, dried over Na_2SO_4 and the solvent evaporated in vacuo to leave a viscous oil.

Reaction of N-aminophthalimide with methyl 10-undecynoate (I). The reaction of N-aminophthalimide (0.6 mmol) with I (3 mmol) in the presence of LTA (0.66 mmol) was carried out as described above. The crude product obtained after workup was chromatographed on a silica gel (25 g) column (11 mm in diameter, effective length 36.5 cm). Elution with a mixture of petroleum ether/ether (78:22, v/v; each fraction 15 ml, total volume 210 ml) gave IV as pale yellow solid (29%) mp 55 C. Found: C, 82.10; H, 8.20; N, 9.52; $C_{20}H_{24}O_4N_2$ requires: C, 82.16; H, 8.27, N, 9.58%. IR(KBr): 1775, 1740–1680, 1600, 1455, 1420, 1370, 1300, 1170, 1070, 880 and 705 cm^{-1}. NMR ($CDCl_3$): δ1.38 (br, s, chain-C$\underline{H}_2$), 2.22 (m, 4H), 3.64 (s, 3H), 4.65 (m, 1H), 7.92 (m, 4H), 8.57 (m, 1H). MS: m/e 358 (M+2, 1), 357 (M+1, 7), 356 (M^+, 27), 355 (M-1, 8), 330 (4), 329 (17), 325 (6), 324 (26), 306 (2), 280 (3), 278 (3), 267 (37), 265 (38), 241 (8), 228 (13), 227 (8), 213 (12), 210 (10), 201 (66), 200 (3), 199 (5), 188 (75), 187 (63), 184 (30), 175 (18), 163 (45), 162 (41), 148 (41), 147 (53), 135 (19), 130 (59), 105 (46), 104 (100), 97 (100), 76 (70) and 74 (23).

Reaction of N-aminophthalimide with methyl 9-undecynoate (II). Similarly, reaction of N-aminophthalimide (0.6 mmol) with II (3 mmol) gave a crude product that was chromatographed on a silica gel (25 g) column (11 mm in diameter, effective length 36.5 cm). Elution with petroleum ether/ether (74:26, v/v; each fraction 15 ml, total volume 225 ml) gave V as a yellow solid (23%) mp 150 C. Found: C, 82.11; H, 8.21; N, 9.53; $C_{20}H_{24}N_2O_4$ requires: C, 82.16; H, 8.27; N, 9.58%. IR(KBr): 1774, 1740–1680, 1600, 1450, 1420, 1370, 1170, 1080, 880 and 715 cm^{-1}. NMR ($CDCl_3$): δ1.33 (br, s, chain—C$\underline{H}_2$—), 1.7 (s, 3H), 2.25 (m, 2H), 2.7 (s, 3H), 3.0 (t, 2H), 3.65 (s, 3H), 7.81 (m, 4H). MS: m/e 358 (M+2, 1), 357 (M+1, 2), 356 (M^+, 3), 341 (2), 325 (2), 324 (3), 304 (3), 297 (3), 279 (8), 266 (3), 265 (3), 243 (5), 229 (4), 227 (4), 213 (6), 201 (25), 199 (8), 189 (16), 188 (25), 187 (16), 169 (18), 167 (10), 163 (20), 162 (20), 149 (38), 148 (23), 147 (45), 130 (29), 105 (29), 104 (66), 97 (31), 76 (55), 74 (26) and 43 (100).

Reaction of N-aminophthalimide with methyl 9-octadecynoate (III). Treatment of III (3.0 mmol) with N-aminophthalimide (0.6 mmol) yielded a crude product that was chromatographed on a silica gel (30 g) column (11 mm in diameter, effective length 45.5 cm). Elution with petroleum ether/ether (80:20, v/v; each fraction 15 ml, total volume 210 ml) gave VI as a yellow solid (19%), mp 132 C. Found: C, 82.91; H, 9.5; N, 6.91; $C_{27}H_{38}N_2O_4$ requires: C, 83.02; H, 9.8; N, 7.02%. IR(KBr): 1775, 1740–1670, 1600, 1455, 1430, 1370, 1190, 1170, 880, 750 and 720 cm^{-1}. NMR (CCl_4): δ0.87 (t, 3H), 1.37 (br, s, chain—C$\underline{H}_2$), 2.24 (t, 2H), 3.01 (t, 2H), 3.6 (s, 3H), 7.76 (m, 4H). MS: m/e 456 (M+2, 0.2), 455 (M+1, 0.5), 454 (M^+, 1), 453 (M-1, 1), 423 (1), 422 (3), 396 (1), 395 (0.3), 394 (0.5), 368 (1), 355 (2), 341 (3), 330 (2), 311 (1), 297 (4), 250 (3), 248 (4), 242 (2), 227 (2), 221 (5), 213 (3), 211 (5), 189 (11), 163 (43), 148 (16), 147 (13), 132 (45), 104 (100), 97 (10), 76 (38) and 74 (10).

ACKNOWLEDGMENTS

M. S. Ahmad, Chairman, Department of Chemistry, provided research facilities. M. H. Ansari received a Senior Research Fellowship from the Council of Scientific and Industrial Research, New Delhi. This research was financed in part by a grant from the USDA under PL-480.

REFERENCES

1. Siddiqui, M.A., F. Ahmad and S.M. Osman, *J. Chem. Research* (S) 26 (1984).
2. Siddiqui, M.A., F. Ahmad and S.M. Osman, *J. Chem. Research* (M) 111 (1984).
3. Breslow, R., *Angew Chem. Int. Ed. Eng. 7*:565 (1968).
4. Breslow, R., J. Brown and J.J. Gajewski, *J. Am. Chem. Soc. 89*:4383 (1967).
5. Neber, P.W., and A. Burgard, *Ann. Chem. 493*:381 (1932).
6. Isomura, K.M., M. Okada and H. Taniguchi, *Tetrahedron Lett. 46*:4073 (1969).

7. Foglia, T.A., P.A. Barr and G.J. Maerker, *JAOCS 49*:414 (1972).
8. Anderson, D.J., T.L. Gilchrist, G.E. Gymer and C.W. Rees, *J. Chem. Soc.* (D) 1518 (1971).
9. Anderson, D.J., T.L. Gilchrist, G.E. Gymer and C.W. Rees, *J. Chem. Soc. Perkin Trans. 1*, 550 (1973).
10. Hassner, A., and F.W. Fowler, *J. Org. Chem. 33*:2686 (1968).
11. Kannan, R., M.W. Roomi, M.R. Subbaram and K.T. Achaya, *Fette Seifen Anstrichm. 69*:644 (1967).
12. Ames, D.E., and R.E. Bowman, *J. Chem. Soc. 677* (1952).
13. Drew, H.D.K., and H.H. Hatt, *J. Chem. Soc. 16* (1937).

[Received May 15, 1985]

A Capillary Gas Chromatographic Method for the Characterization of Linear Fatty Alcohols

Robert E. Oborn and **Alan H. Ullman***
The Procter & Gamble Company, Cincinnati, Ohio

A capillary gas chromatography (GC) method for the analysis of fatty alcohols is described. The method can separate fatty alcohols, fatty acids, hydrocarbons and fatty acid methyl esters containing 6 to 22 carbons, as well as fatty-fatty esters to 40 or more carbons. The precision of the method is better than 2% (rsd); accuracy, based on analyses of a standard mixture and a spiking/recovery experiment, is better than 3% (relative difference between known and measured). A calculated hydroxyl value based upon the GC data agrees well with the titrimetric hydroxyl value.

Gas chromatography is undoubtedly the most important analytical technique for the characterization of fatty alcohols (C_6 to C_{18} alkanols). The technique provides chain length and purity information, and hydroxyl value and compositional data, all at the same time. However, most published methods for the GC analysis of fatty alcohols utilize packed columns which do not provide sufficient resolution for many applications. Some of the liquid phases which have been reported include silicones such as SE-30 (1,2); OV-1 (3); OV-7 (2); OV-17 (3,4); OV-225 (3,4); Silar-10C (2,4); Silar 5-CP (2,5), and glycols such as PEG 4000 (6) and PEGS (7). In many cases the sample is analyzed as an ester or silyl ether derivative (5). The method in the U.S. Pharmacopeia/National Formulary (8) for cetostearyl alcohol uses a methyl silicone gum.

None of these methods provides the high resolution of a modern programmed temperature capillary column technique. The benefits of such an approach include separation of alcohols, methyl esters, hydrocarbons and related fatty materials. Korhaven (9,10) has demonstrated the separation of alcohols and several kinds of esters using SE-30 and OV-351 wall-coated open tubular capillary columns.

We report here a capillary GC method for industrially important linear fatty alcohols which resolves most of the compounds likely to be found in such materials. As an added benefit the method can be used to calculate the hydroxyl value.

EXPERIMENTAL

Instrumentation and operating conditions. GC conditions are summarized in Table 1. Three different GC's were used during the course of this work; results were comparable. The DB-1 capillary column had about 50,000 theoretical plates based on the "peak width at half height" approximation (11).

To establish maximum sensitivity of the flame ionization detectors, injections of octadecanol were made at different hydrogen to air ratios. Air levels of 300–500 ml/min at 50 ml/min in 5 ml/min increments were tested while the hydrogen was varied from 25 ml/min to 45 ml/min in 5 ml/min increments. Maximum peak area for the octadecanol sample was obtained at the gas flows listed in Table 1.

Virtually identical results were obtained with either helium or nitrogen as carrier gas.

The injection port temperature initially was set at 360 C to ensure the vaporization of high molecular weight species such as the fatty-fatty or wax esters (e.g. stearylstearate). Subsequent experiments demonstrated equally good results with a lower injection temperature (280 C). In fact, at 360 C there appeared to be some

TABLE 1

GC Conditions Used for the Analysis of Fatty Alcohols

Instruments	P-E 910 (Perkin-Elmer, Norwalk, Connecticut) H-P 5840, H-P 5880 (Hewlett-Packard, Avondale, Pennsylvania)
Injection port temperature	280 C
Flame ionization detector (FID) temperature	360 C
Column oven temperature	From 75 C to 300 C at 10 C/min, then 300 C for 5 min
Air flow to FID	400 ml/min
Hydrogen flow to FID	30 ml/min
Carrier gas flow	1 ml/min
Split ratio	100:1
Carrier gas	Helium or Nitrogen
Column	15 m × 0.24 mm ID fused silica with 0.25 micron coating of DB-1 (J&W Scientific, Rancho Cordova, California)
Attenuation	16

*To whom correspondence should be addressed at The Procter & Gamble Company, 6250 Center Hill Rd., Cincinnati, OH 45224.