Uchebn. Zaved Pishch. Tekhnol. 4:37 (1975).
4. Labuza, T.P., H. Tsuyuki and M. Karel, JAOCS 46:409 (1969).
5. Witting, L.A., Arch. Biochem. Biophys. 129:142 (1969).
6. Lloyd, W.G., J. Polym. Sci. A_1 2551 (1963).
7. Hendry, D.C. and G.A. Russel, J. Am. Chem. Soc. 86:2368 (1964).
8. Uri, N., Nature 177:1177 (1956).
9. Cohen, G. and R.E. Heikkila, J. Biol. Chem. 249:2447 (1974).
10. Rogstad, A. and R. Reinton, JAOCS 54:282 (1977).
11. Ke, P.J. and R.G. Ackman, Ibid. 53:636 (1976).
12. Farag, R.S. and S.A. Osman, Ibid. 55:703 (1978).
13. Gruger, E.H. and A.L. Tappel, Lipids 5:326 (1969).
14. Cillard, J., Doctorate thesis in Pharmaceutical Sciences, Rennes, June 1978.
15. Morita, M., M. Mukunoki, F. Okubo and S. Tadokoro, JAOCS 53:489 (1976).

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Fatty Acids: XX[1]. Location of the Position of the Furan Ring in 2,5-Disubstituted Furan-containing Fatty Acids by GLC Analysis of Oxidation Products

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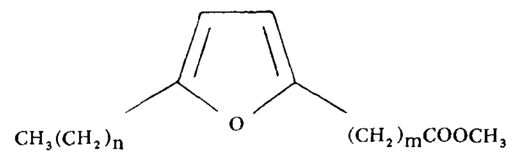
ABSTRACT

Oxidation of 2,5-disubstituted furan-containing fatty acids by the von Rudloff or Brown procedures results in the production of monoacid and diacid fragments, which can be readily identified by gas liquid chromatography (GLC) analysis. Cleavages occur at the double bonds of the furan ring system with von Rudloff's reagent. A mixture of 2 monoacid and 2 diacid fragments are obtained instead when the furan-containing fatty acid is treated with chromic acid according to Brown's procedure. These chemical methods are suitable for locating the position of the furan system in the fatty acid chain.

INTRODUCTION

Furan-containing fatty acids have been isolated from seed oil (1) and fish oils (2-6), and were more recently found in high proportion (90%) in the triglyceride fraction of the latex of the rubber tree *(Heavea brasiliensis)* (7). The role of these fatty acids in nature remains vague, but they are believed to be associated with sexual maturity in the fish as these compounds occur in high concentrations in the testes during spawning time.

The author has reported the synthesis of a complete series of 2,5-disubstituted furan fatty acids (8). Some of the chromatographic properties have already been reported (9,10), whereas the spectrometric behavior is currently being assessed (Lie Ken Jie, M.S.F., unpublished results). Mass spectrometric analyses of the various furan fatty acid isomers have clearly shown a characteristic mass fragmentation pattern, which permits the location of the furan ring system in the fatty acid chain (1,8,11,12). We now report a ready chemical method for the determination of the furan ring position by gas liquid chromatography (GLC) analysis of the oxidation products when the furan-containing fatty acid is treated either with von Rudloff's oxidant or chromic acid. The following 4 positional isomers of 2,5-disubstituted C_{18} furan fatty esters were selected for this study:



n	m
8	4
7	5
3	9
1	11

EXPERIMENTAL PROCEDURE

Modified von Rudloff Oxidative Cleavage Procedure (13)

A mixture of *tert*-butanol (40 ml), 2% aqueous potassium carbonate (7 ml), oxidant (14 ml, prepared by dissolving 0.2 g potassium permanganate and 10.4 g sodium periodate in 500 ml of water) and furan fatty ester (25 mg) contained in a 100-ml stoppered round-bottomed flask was mechanically stirred at room temperature for 18 hr. The light-purple-colored mixture faded only slightly after this reaction period. Sulfur dioxide gas was bubbled through the solution until a persistent light-yellow solution was obtained. Potassium hydroxide pellets (15 pellets = ∿2 g) were added and the mixture swirled until all potassium hydroxide pellets were dissolved. A 2-phase mixture was obtained and a drop of this basic mixture was removed and tested with litmus or universal indicator paper. If the mixture was found to be acidic, more potassium hydroxide was added until a strongly basic solution/mixture was obtained. The mixture was then carefully evaporated under reduced pressure (∿40 mm Hg) using a rotary evaporator at a bath temperature of 70 C until no solvent remained. A white powder was left in the flask, which was acidified with 2 M HCl (30 ml). A 30% sodium chloride solution (10 ml) was added and the resulting mixture extracted with diethyl ether (3 x 20 ml). The ethereal extract was washed with 30% sodium chloride solution (2 x 20 ml) and dried over anhydrous sodium sulfate. The ether was carefully distilled on a water bath (50 C) and the residue treated with

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[1] Part XIX by M.S.F. Lie Ken Jie appeared in J. Chromatog. 192:457 (1980).

anhydrous methanol (20 ml) and 14% BF_3-methanol complex (5 ml). The methanolic solution was refluxed for 10 min under anhydrous conditions. The reaction mixture was then cooled, sodium chloride solution (50 ml) was added and the resulting mixture extracted with light petroleum (bp 40-60 C, 3 x 20 ml). The petroleum extract was washed with sodium chloride solution (2 x 10 ml) and dried over anhydrous sodium sulfate. The filtered solution was distilled and the residue contained in 1-2 ml of petroleum was transferred into a stoppered test tube (5-ml size) for GLC analysis.

Brown's Oxidative Cleavage Procedure (14)

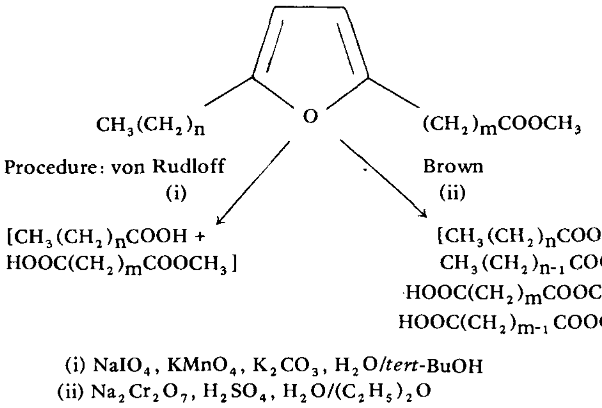
A mixture of furan fatty ester (25 mg) and diethyl ether (10 ml) contained in a 50-ml conical flask was stirred using a magnetic bar stirrer. Chromic acid solution (5 ml, prepared by dissolving 20 g sodium dichromate in 30 ml of water, 28.3 g of concentrated sulfuric acid was added and the mixture diluted to 65 ml with water) was added by drops into the ethereal solution over 10 min. The resulting 2-phase system was stirred vigorously at room temperature for 5 min longer. Water (20 ml) was added to the reaction mixture and the ether layer was isolated and washed with 30% sodium chloride solution (3 x 5 ml) and dried over anhydrous sodium sulfate. The solvent was carefully distilled on a 50 C water bath and the residue esterified using BF_3-methanol complex as already described. The esterified product was subjected to GLC analysis.

GLC Conditions

A Pye Model 104 gas chromatograph was used equipped with glass columns (2 m, 6 mm diameter) containing 15% Silar 10C on Chromosorb W (80-100 US mesh) or 1.5% OV-101 on Chromosorb W (80-100 US mesh) with a nitrogen flow rate of 40 ml/min. The Silar 10C column was maintained at 120 C for the monocarboxylic ester fragments and at 160 C for the dicarboxylic esters, whereas for the OV-101 column a constant temperature of 120 C was employed. Methyl decanoate, laurate, palmitate and diesters of adipic, suberic and azelaic acid were used as internal reference standards.

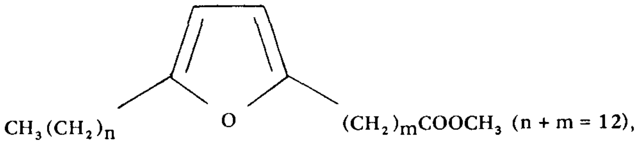
RESULTS AND DISCUSSION

The oxidation products of the C_{18} furan fatty esters by the von Rudloff and Brown procedures are shown in Scheme I. A distinct difference in these 2 methods of oxidation, besides the use of different reagents, was apparent in the acidic or basic conditions in which each of these reactions was conducted. The von Rudloff procedure was carried out at a slightly basic condition, pH ca. 8, by the addition of a small amount of potassium carbonate, whereas the Brown method was performed under a very acidic medium, pH ca. 1, by addition of chromic acid and sulfuric acid to the substrate dissolved in diethyl ether.



SCHEME I. Oxidative cleavage of furan fatty esters.

The results of the GLC analyses of the monoacid and diacid fragments isolated after the oxidation of furan fatty esters implied that cleavage occurred at the C=C bonds between the 2,3- and 4,5-positions of the furan ring during von Rudloff's oxidation. Thus from the furan fatty ester,



the monoacid and diacid fragments obtained from the von Rudloff oxidative cleavage procedure were $CH_3(CH_2)_n$-COOH and $HOOC(CH_2)_mCOOCH_3$, respectively. Difficulties were experienced in the isolation of the monoacid fragments in 2 of the positional isomers of the C_{18} furan fatty acids studied, where the alkyl chains were significantly short ($n \leqslant 3$); however, isolation and identification of the corresponding diacid fragments were readily achieved.

Chromic acid oxidation of furan fatty esters according to the Brown procedure resulted in the isolation and identification of 2 monoacid and 2 diacid fragments instead. The acidic condition in which such reactions took place apparently caused ring opening of the furan system to yield the corresponding dimethylene interrupted dioxo derivatives (Scheme I). Subsequent interaction of the derivatives with chromic acid allowed oxidative cleavages to occur between the C-C bonds on either side of the oxo functions in the alkyl chain. The 2 monoacid [$CH_3(CH_2)_n$-COOH and $CH_3(CH_2)_{n-1}COOH$] and diacid [HOOC-$(CH_2)_mCOOCH_3$ and $HOOC(CH_2)_{m-1}COOCH_3$] fragments were readily identified by GLC analyses. Again, where $n \leqslant 3$, the monoacid fragments could not be isolated for GLC analysis, but the diacid fragments unequivocally confirmed the chain length of the furan substituents.

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REFERENCES

1. Morris, L.J., M.O. Marshall and W. Kelley, Tetrahedron Lett. 36:4249 (1966).
2. Glass, R.L., T.P. Krick and A.E. Eckhardt, Lipids 9:1004 (1974).
3. Glass, R.L., T.P. Krick, D.M. Sand, C.H. Rahn and H. Schlenk, Ibid. 10:695 (1975).
4. Glass, R.L., T.P. Krick, D.L. Olson and R.L. Thorson, Ibid. 12:828 (1977).
5. Gunstone, F.D., R.C. Wijesundera, R.M. Love and D. Ross, J. Chem. Soc. Chem. Commun. 630 (1976).
6. Scrimgeour, C.M., JAOCS 54:210 (1977).
7. Hasma, H. and S. Subramaniam, Lipids 13:905 (1978).
8. Lie Ken Jie, M.S.F. and C.H. Lam, Chem. Phys. Lipids 21:275 (1978).
9. Lie Ken Jie, M.S.F. and C.H. Lam, J. Chromatogr. 138:373 (1977).
10. Lie Ken Jie, M.S.F. and C.H. Lam, Ibid. 138:446 (1977).
11. Gunstone, F.D. and R.C. Wijesundera, Chem. Phys. Lipids 24:193 (1979).
12. Lie Ken Jie, M.S.F. and C.H. Lam, Ibid 20:1 (1978).
13. von Rudloff, E., Can. J. Chem., 34:1413 (1956).
14. Brown, H.C., C.P. Garg and K.T. Liu, J. Org. Chem., 36:387 (1971).

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