CHROM. 5822

Gas chromatography of cyclopropane fatty acid methylesters prepared with methanolic boron trichloride and boron trifluoride*

CHRISTIE[1] has suggested that the use of boron trichloride in methanol (BCl_3–CH_3OH) by BRIAN AND GARDNER[2–4] for esterification of bacterial fatty acids may have resulted in unreliable gas chromatographic (GC) data. MINNIKIN AND POLGAR[5] showed that methanolic boron trifluoride (BF_3–CH_3OH) reacted with disubstituted cyclopropanes to give methoxyesters and the corresponding olefins. The possibility that a similar and unnoticed side-reaction may have occurred when BCl_3–CH_3OH was used to esterify cyclopropane fatty acids of bacterial origin[3,4] was implied[1].

BF_3 has been shown to be superior to BCl_3 as a catalyst for fatty acid transesterification[6]. Detailed studies using BF_3 or BCl_3 in CH_3OH for esterification[6–8] did not include lipids containing cyclopropane fatty acids. A large number of papers have been published in which BF_3 or BCl_3 was used to catalyze the reaction of CH_3OH with bacterial fatty acids. Few authors who used BF_3 or BCl_3 in CH_3OH have indicated that another procedure was employed to check recovery of cyclopropane acids from bacterial lipids[4,9].

The purpose of this investigation was to determine the reliability of GC data following use of commercially available BF_3–CH_3OH and BCl_3–CH_3OH in the esterification of bacterial fatty acids containing cyclopropane rings.

Experimental

Esterification of fatty acid standards. A standard solution was prepared containing 1 mg/ml each of *cis*-9,10-methylene octadecanoic (cyc C_{19}) acid (Supelco, Inc., Bellefonte, Pa.) and heptadecanoic (C_{17}) acid (Applied Science Laboratories, State College, Pa.) in chloroform ($CHCl_3$).

1-ml aliquots were transferred to 15 × 150 mm screw-cap tubes and $CHCl_3$ was evaporated with a stream of N_2 (30°). 2 ml of 14% BF_3 in CH_3OH (w/v) or 10% BCl_3 in CH_3OH (w/v), both from Applied Science Laboratories, were added and the open tube placed in boiling water for 2 min (ref. 7). The tube was cooled and the contents transferred to a 30-ml separatory funnel. The tube was washed with 4 ml of $CHCl_3$, the $CHCl_3$ plus 1 ml of water were added to the separatory funnel and the contents shaken and allowed to separate. The $CHCl_3$ phase containing methylesters was evaporated with N_2 in a screw-cap tube.

The fatty acid standards were also esterified in an open tube for 0.5 min (100°), and for 5 min (100°) while tightly closed by a teflon-lined screw-cap.

Gas chromatography of fatty acid methylesters. Dried methylesters were dissolved in 1 ml of $CHCl_3$ and 1 μl was injected into an Aerograph (Varian Associates, Palo Alto, Calif.; Model 204-1C) gas chromatograph with flame ionization detectors. Columns (5 ft. × 1/8 in.) containing 15% diethylene glycol succinate polyester on Chromosorb W (60–80 mesh) were operated at 180°. Detector and injector temperatures were 220° and the N_2 flow was 25 ml/min. Range was 10^{-10} and attenua-

* This work was supported by Faculty Research Funds of North Texas State University and by Robert A. Welch Foundation, Grant B-268.

tion was 8. Areas of peaks were calculated by multiplication of peak height by peak width at 1/2 height.

Esterification of bacterial fatty acids. Escherichia coli (ATCC 11775) was incubated for 16 h at 40° in Trypticase Soy Broth (BBL). Higher incubation temperature is known to favor cyclopropane fatty acid production[10]. Lipids were extracted with $CHCl_3$–CH_3OH (2:1) (ref. 11). A solution containing 1 mg/ml C_{17} and 1.2 mg/ml *E. coli* lipid in $CHCl_3$ was esterified with BCl_3–CH_3OH and BF_3–CH_3OH by the method of METCALFE, SCHMITZ, AND PELKA[8]. Fatty acids were identified by hydrogenation, bromination[3] and by comparison with authentic standards. Cyclopropane methylesters (*cis*-9,10-methylene hexadecanoate, cyc C_{17}, and *cis*-11,12-methylene octadecanoate, cyc C_{19}) were synthesized from palmitoleate and *cis*-vaccenate using a simplified zinc–copper couple[12] and the SIMMONS–SMITH reaction[13].

Results and discussion

Results of BF_3–CH_3OH and BCl_3–CH_3OH were compared with the reaction of 2 ml of freshly distilled diazomethane at 0° for 30 min (Table I). Diazomethane gave quantitative recovery of cyc C_{19} (99–101%). Recovery of cyc C_{19} (retention time relative to C_{17} = 2.03) using BCl_3–CH_3OH at 100° for 2 min (open tube) or 5 min (closed tube) was similar to that obtained with diazomethane (93–100%). BF_3–CH_3OH gave poor recovery of cyc C_{19} (10–50%) depending on conditions of the reaction (see Table I). Similar results were obtained with a 10% solution of BF_3 in CH_3OH prepared from a BF_3–ether complex, $BF_3 \cdot O(C_2H_5)_2$ (Eastman Chemical Co.).

TABLE I

GC RESULTS OF FATTY ACID METHYL ESTERS FOLLOWING VARIOUS ESTERIFICATION METHODS

Fatty acids: *cis*-9,10-methylene octadecanoic (cyc C_{19}) acid and heptadecanoic (C_{17}) acid (internal standard); esterification: 1 mg of each acid; results (range of 3 determinations); peak areas for fatty acid methyl esters relative to C_{17} taken as 1.00.

Esterification method	*Relative peaks areas*	
	cyc C_{19}	*Other esters*
Diazomethane, 30 min, 0°	0.99–1.01	
Open tube, 0.5 min, 100°		
BF_3–CH_3OH	0.45–0.59	0.14–0.15
Open tube, 2 min, 100°		
BCl_3–CH_3OH	0.93–1.00	
BF_3–CH_3OH	0.12–0.13	0.31–0.32
Closed tube, 5 min, 100°		
BCl_3–CH_3OH	0.96–0.98	
BF_3–CH_3OH	0.10–0.11	0.46–0.50

Five additional peaks (retention times relative to C_{17} = 1.40, 1.64, 1.83, 3.12 and 4.23) were obtained when BF_3–CH_3OH was used (Table I).

Results of BCl_3–CH_3OH for esterification of *E. coli* fatty acids (Fig. 1) indicated that cyc C_{17} comprised 29% and cyc C_{19} 16% of the total acids. Chromatograms obtained, following use of BF_3–CH_3OH (Fig. 1), revealed considerable loss of cyclopropane esters as well as additional small peaks. Therefore, BF_3–CH_3OH appears

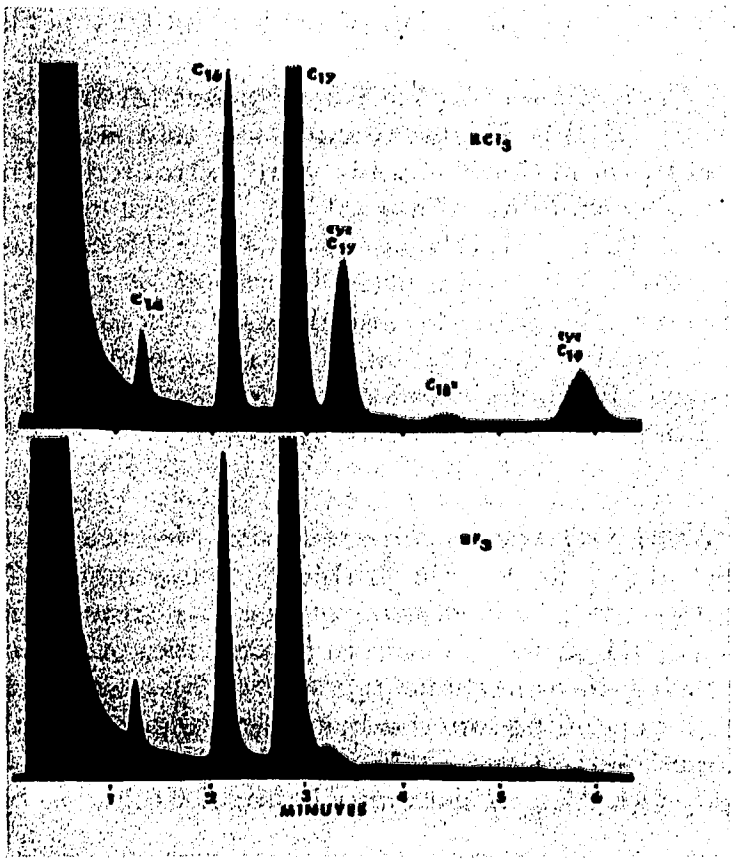

Fig. 1. Authentic gas chromatograms of methylesters of *Escherichia coli* fatty acids with C_{17} internal standard (areas under the peaks are darkened). Esters were prepared[8] using BCl_3–CH_3OH (upper) and BF_3–CH_3OH (lower).

to be an undesirable esterification reagent for fatty acid mixtures containing cyclopropanes. BCl_3–CH_3OH on the other hand, is suitable for this purpose and appears to quantitatively esterify cyclopropane fatty acids which are prevalent in the lipids of many bacterial species.

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Received October 25th, 1971