Artifacts produced by boron trifluoride methanolysis of a synthetic lecithin containing cyclopropane fatty acids (1-2-dihydrosterculoyl-3-*sn*-phosphatidylcholine)

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SUMMARY It is shown that methanolysis of dihydrosterculoyl lecithin with boron trifluoride–methanol introduces artifacts which are absent if the methyl ester is prepared by saponification of the lipid followed by treatment with diazomethane.

SUPPLEMENTARY KEY WORDS gas–liquid chromatography · diazomethane

Methanolysis of lipids with boron trifluoride is widely used in the determination of fatty acid composition by gas–liquid chromatography (1). The production of an artifact from oleic acid by this procedure has recently been reported (2), and it is also known that long-chain cyclopropane esters are attacked by boron trifluoride–methanol to give methoxy esters (3). In our studies with synthetic phospholipids we have observed that methylation by this method produces a group of artifacts when applied to lipids containing dihydrosterculic acid, a cyclopropane fatty acid. Cyclopropane fatty acids are known to be components of several bacterial membranes. For example, in lactobacilli and *Agrobacterium tumefaciens*, *cis*-11,12-methyleneoctadecanoic acid is a major lipid constituent, and dihydrosterculic (*cis*-9-10-methylenehexadecanoic) acid is a component of *Escherichia coli* membranes in the late logarithmic phase of growth (4). Since considerable interest has recently been focused on the relationship between the lipid composition and physiological functions of bacterial membranes (5, 6), it seems important to call attention to this limitation of boron trifluoride methanolysis.

A sample of synthetic dihydrosterculoyl lecithin, shown to be a pure phosphatidylcholine by thin-layer chromatography, was methylated with boron trifluoride–methanol (14% [w/v], obtained from Applied Science Laboratories Inc., State College, Pa.) according to the method of Morrison and Smith (1). The resulting product was then analyzed without further purification on a

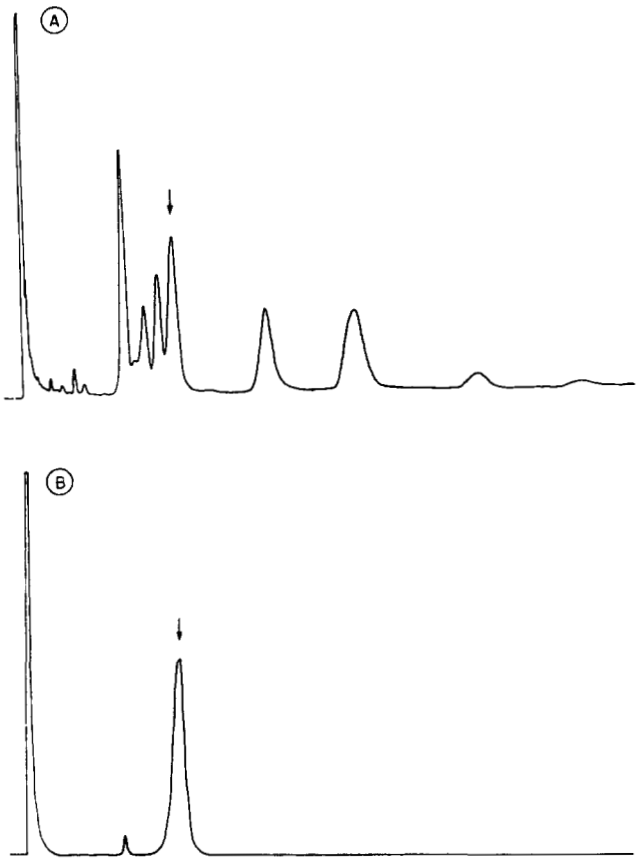

FIG. 1. *A*, gas–liquid chromatogram of products formed by boron trifluoride methylation of synthetic dihydrosterculoyl phosphatidylcholine. *B*, gas–liquid chromatogram of products formed by methylation with diazomethane of saponified dihydrosterculoyl phosphatidylcholine. The arrows in *A* and *B* indicate the position corresponding to the retention time of an authentic sample of methyl dihydrosterculate.

Beckman GC-4 gas chromatograph equipped with flame ionization detectors. Columns (6 ft × $^1/_8$ in.) consisting of 15% EGSS-X on 100–200 mesh Gas-Chrom P (Applied Science Laboratories) were operated at 165°C with nitrogen as the carrier gas. The chromatogram shown in Fig. 1*A* was obtained. Similar results were achieved with other samples of the lecithin, and did not seem to vary with different batches of boron trifluoride–methanol. Attempts to identify the peaks in the chromatogram by comparison with commonly available standard methyl esters were unsuccessful. However, when the dihydrosterculoyl lecithin was saponified with potassium hydroxide then methylated with diazomethane (7) and analyzed without further purification, the chromatogram shown in Fig. 1*B* was obtained.

Methylation of a sample of dihydrosterculic acid (Analabs Inc., North Haven, Conn.) by the two procedures described above gave results qualitatively similar to those obtained with the phospholipid. Furthermore, the retention time of the single methyl ester formed by diazomethane from both the lecithin and the free acid agreed with that of authentic methyl dihydrosterculate.

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