Separation and isolation of methyl esters and dimethylacetals formed from brain lipids

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The procedure of Stoffel, Chu, and Ahrens (1) for preparing and separating the methyl esters of fatty acids and the dimethylacetals (DMA) when applied to brain lipid extracts proved adequate for the nonoxygenated fatty acids and DMA. Some of the cholesterol in the extract, however, was converted to derivatives, one of which, Δ5,6-cholesten-5-one (2), sublimed along with the methyl esters and the DMA. Furthermore, it was found that under the conditions specified by Stoffel et al. (1), approximately 75% of the 2-hydroxy methyl esters remained unsublimed.

Reports (3–5) on the fractionation and estimation of nonoxygenated and oxygenated fatty acid derivatives suggest that a thin-layer chromatographic procedure (TLC) could be developed to separate quantitatively the esters of the nonoxygenated and of the 2-hydroxy fatty acids as well as of the DMA. This consideration and the possibility that cholesterol and its derivatives could be separated were explored.

Chimyl alcohol, glyceryl monostearate, 12-hydroxy stearic acid, behenic acid, 2-hydroxy behenic acid, oleyl and behenyl alcohols, nonoxygenated methyl esters,

1 Abbreviations used: Fatty acids are identified by their carbon number and number of double bonds. An h indicates the presence of a 2-hydroxy group.
cholesterol and tetra- and octadecanal were obtained commercially. The 2-hydroxy methyl esters, C18h:0, C22h:0, and C24h:0, were a generous gift of Dr. J. F. Mead.

The procedures for the extraction of lipids and the determination of carboxyl esters and aldehydes have been reported previously (2). The fatty acids and DMA were methylated in sealed tubes according to Farquhar (6).

Chromatoplates, 20 × 20 cm, were coated with layers, 250-275 μ thick, of Silica Gel G (Research Specialties Co., Richmond, Calif.), using the Desaga-Brinkmann equipment (Brinkmann Instruments Co., Great Neck, N.Y.) according to Stahl (7). The plates were activated at 100° for 2 hr. The chromatographic chamber was lined with Whatman No. 1 filter paper and flushed once with nitrogen after the introduction of 200 ml of developing solvent. The solvents used were petroleum ether (bp 40–60°)-diethyl ether 60:40 (v/v) (8) and xylene, which were redistilled from an all-glass still within 24 hr prior to use. These solvents were allowed to equilibrate in the chamber for 1 hr before development of the chromatoplates. The entire chromatographic procedure was performed without direct exposure to light.

Gas-liquid chromatography (GLC) was done on a Barber-Coleman Model 10 apparatus equipped with a radium ionization detector as described elsewhere (2).

The chromatoplates were marked with the aid of the Brinkmann labeling template. The points of origin started 3 cm from the left edge and 2.5 cm from the base of the plate. Exactly 9 cm above the origin, a 5-mm line was drawn parallel to the base at the left and right edge of the plate. Fifteen centimeters above the origin, a line was drawn parallel to the base across the entire plate.

A solution of methylation products obtained from a brain lipid extract containing 10–20 μmoles of methyl esters was placed in a 13 x 100-mm test tube and concentrated to 0.2–0.3 ml with a stream of nitrogen. The entire sample was applied with a micropipette to a chromatoplate in a row of 30 spots, about 5 mm apart. The plate was first developed in the petroleum ether–diethyl ether mixture until the solvent front had ascended close to the origin. Consecutive development of the chromatoplate with petroleum ether-diethyl ether and xylene (Table 1) accomplished separation of the DMA, nonoxygenated methyl esters, and 2-hydroxy methyl esters from each other, and from cholesterol and its major derivatives. Some cholesterol derivatives, however, migrated to the same areas as the DMA, and the 2-hydroxy methyl esters were not separated from alcohols of similar chain lengths.

Employing the petroleum ether–diethyl ether or the xylene solvent system for quantities between 0.8 and 22 μmoles, the recoveries of the nonoxygenated methyl esters was 95%. The nonoxygenated methyl esters and DMA from the 2-hydroxy methyl esters, long chain alcohols, cholesterol, and the major cholesterol derivative (Table 1). The methyl esters of the nonoxygenated fatty acids, however, were not separated from the DMA. While development with xylene did separate the nonoxygenated methyl esters from the DMA (Table 1), the 2-hydroxy methyl esters and cholesterol remained close to the origin. Consecutive development of the chromatoplate with petroleum ether–diethyl ether and xylene (Table 1) accomplished separation of the DMA, nonoxygenated methyl esters, and 2-hydroxy methyl esters from each other, and from cholesterol and its major derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Solvent System</th>
<th>A*</th>
<th>B†</th>
<th>C‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonoxygenated methyl esters</td>
<td>Petroleum ether–diethyl ether 60:40 (v/v)</td>
<td>0.78</td>
<td>0.43</td>
<td>0.64</td>
</tr>
<tr>
<td>2-Hydroxy methyl esters</td>
<td></td>
<td>0.41</td>
<td>0.05</td>
<td>0.28</td>
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<tr>
<td>12-Hydroxy methyl stearate</td>
<td></td>
<td>0.41</td>
<td>0.05</td>
<td>0.26</td>
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<tr>
<td>Dimethylacetals</td>
<td></td>
<td>0.78</td>
<td>0.15</td>
<td>0.53</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td>0.20</td>
<td>0.05</td>
<td>0.15</td>
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<tr>
<td>Cholestaifene</td>
<td></td>
<td>0.90</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>Glyceryl monostearate</td>
<td></td>
<td>0.03</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Chinyl alcohol</td>
<td></td>
<td>0.03</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Oleyl alcohol</td>
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<td>0.40</td>
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<td>0.26</td>
</tr>
<tr>
<td>Behenyl alcohol</td>
<td></td>
<td>0.40</td>
<td>0.05</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* Petroleum ether–diethyl ether 60:40 (v/v).
† Xylene.
‡ Consecutive development with petroleum ether–diethyl ether and xylene.
§ RF values given for each solvent system were determined on a single plate.
estimers ranged from 97 to 99%, that of the oxygenated methyl esters (C22h:0 and C24h:0) ranged from 94
to 98%. After consecutive development with these solvent
systems, the recoveries of the individual non-
oxogenated methyl esters were 99% for C18:1, C20:1,
and C24:1; 94–99% for C22:5; and 92–99% for C22:6.
A recovery of 99% was obtained for a mixture of C18:1,
C20:1, C20:5, C22:6, and C24:1. Analysis of this
mixture by GLC before and after TLC showed an es-
tentially identical percentage composition. The re-
covery of DMA of tetradecanal and octadecanal
was 99% for C18:0, C22:0, and C24:0. After
consecutive development with these sol-
vent systems, the recoveries of the individual non-
oxygenated methyl esters ranged from 94
to 99%. It averaged 85% for 33 plates, and its repro-
ducibility on any particular lipid sample was generally
within 5%; thus, lower recovery was characteristic of
certain lipid samples rather than of the TLC procedure.
Reasons for low recovery of DMA from these extracts
have not been ascertained as yet.

Recoveries from a synthetic mixture of purified methyl
esters and DMA (Table 2) on four plates were 102–105% for
the nonoxygenated esters, 89–107% for the 2-hydroxy
methyl esters, and 90–96% for the DMA. Recoveries of
methyl esters from brain lipid extracts on 37 plates
varied between 89 and 102%, with an average of 99%.
In one sample, an unexplained recovery of 114% was
noted. The percentage deviation for replicate analyses
of 2-hydroxy fatty acids was 2.4%. These data were based
upon 14 duplicates, 3 triplicates, 1 quadruplicate, and 2
quintuplicate experiments.

The procedure presented is simple, rapid, and ap-
licable for studying the fatty acids from brain and pos-
sibly other tissue lipid extracts. The results suggest that
the DMA can be reliably analyzed by GLC if the ster-
oids are removed from the lipid extract prior to methyla-
tion.

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REFERENCES