ery test. Therefore, experiments for the complete recovery of added vitamin B_{12} are not necessary in this assay.

The cobalt line is measured against the background of the plate, using a densitometer. To prepare standard synthetic powders, 0.1, 0.2, 0.3, and 0.4 ml. of a standard cobalt solution containing 10 mcg. Co/ml. [49.38 mg. cobalt nitrate, $Co(NO_3)_2 \cdot 6H_2O$, dissolved in 1000 ml. distilled water] are added to four 1-ml. portions of the vitamin solution being analyzed. Each sample is treated as mentioned previously, after mixing with 0.1 g. graphite powder.

An atomic absorption spectrophotometer may be used for this determination. While freedom of interference is better in emission spectrography, because of the higher resolving power of the large spectrographs used, the sensitivities of detection are of the same order of magnitude. Although the atomic absorption method may be superior in rapidity and probably precision, we can mention the following points in favor of emission spectrography:

1. Solid samples can be analyzed directly after drying and grinding with graphite powder.

2. Solvent extraction is not needed for the range of cobalt concentration usually encountered in biological materials.

3. The spectrographic plate is a valuable and permanent record of analytical data, enabling a complete qualitative and quantitative check at any time. This fact is particularly important in legal cases.

In the authors' opinion, emission spectrography and atomic absorption must be used simultaneously and concurrently for the exact determination of traces of metals in biological materials. Their respective results for every metal should doublecheck each other.

In the spectrographic procedure, a "National" carbon cupped electrode, type L 3900, is used as the lower electrode, and a "National" L 3957 is used as the counter electrode. The charge weighed into the cavity is 20 mg. The distance between electrodes, or analytical gap, is 3 mm., which is further increased to 6 mm. during arcing. A d.c. arc, 14-amp., generated from N.S.L. Spec Power,[1] is used as the excitation source. The time of exposure is set to 13 sec., during which up to 3 mcg. of Co in 20 mg. dry residue is completely volatilized. Prearcing is not necessary. A Bausch & Lomb Large Littrow spectrograph is used for this assay, with a fixed slit of 20-μ width and 3-mm. height. The spectrographic plate is Kodak No. 1, size 4 × 10 in., which is developed and dried using a N.S.L. processor. It is measured with a N.S.L. reader (densitometer).

The characteristic curve of the plate is obtained by plotting a preliminary curve, using an iron arc spectrum taken with a two-step filter. This method of plate calibration seems to have a satisfactory precision, because random scatter of experimental points is compensated for in drawing the preliminary curve. The characteristic curve is practically unchanged for a period of laboratory work. Concentrations are plotted against intensities on ordinary graph paper.

[1] National Spectrographic Laboratories Inc., Cleveland, Ohio.

By taking three spectra from each powder, a precision of ±10% was attained.

A large number of vitamin B_{12}-containing preparations such as liver extract and liver injection were analyzed by this method. It was also used to follow separation or purification steps in manufacturing processes (fermentation, extraction, *etc.*). To indicate the precision obtained, we are giving here the results of a routine determination. The analysis of a vitamin B_{12} preparation claimed to contain 40 mcg. vitamin/ml. gave the following results: 1.7, 1.8, 2.0, 2.0, 1.6, and 2.0 mcg. Co/ml. in six different plates. The mean value, 1.85, multiplied by 100/4.35 = 23 gives 42 mcg. cyanocobalamin/ml.

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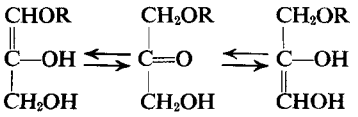
Synthesis of *O*-Alkyldihydroxyacetone and Derivatives

Keyphrases □ *O*-Alkyldihydroxyacetone, derivatives—synthesis □ IR spectrophotometry—structure □ GLC—identity

Sir:

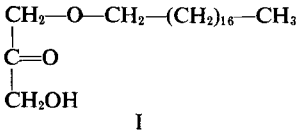
Recently, Snyder and coworkers (1–4) proposed a biochemical pathway and identified intermediates that demonstrated the biosynthesis of *O*-alkyl ether bonds in glycerolipids from fatty alcohols and dihydroxyacetone-P in cell-free systems. This new metabolic scheme included *O*-alkyldihydroxyacetone and *O*-alkyldihydroxyacetone-P as intermediates. We have now further substantiated the previously reported biochemical reaction sequence (3) by the chemical synthesis of one of the intermediates, namely *O*-alkyldihydroxyacetone (I) (R = octadecyl or hexadecyl) (Scheme I). This preliminary communication describes the synthesis of I which can exist as a keto-enol tautomer; the properties of I are identical with those of the ^{14}C-*O*-alkyldihydroxy-



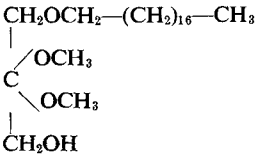
Scheme I

acetone synthesized enzymically by microsomes (4), thereby confirming it as an intermediate in the biosynthesis of these new and metabolically important ether-linked keto lipids.

We have prepared structures with the following general formulas:



I



II

Two synthetic routes were investigated in the synthesis of 1-*O*-octadecyl-2,2-dimethoxy-3-hydroxypropane (II). In the first procedure, acetyl dihydroxyacetone dimethyl ketal was synthesized (5) and then converted to 1-*O*-trityl-2,2-dimethoxy-3-hydroxypropane (III), which had the correct elemental analysis; m.p. 127–128°.

Anal.—Calcd. for $C_{24}H_{26}O_4$: C, 76.16; H, 6.92. Found: C, 76.40; H, 7.01.

IR spectrum: 3500 cm.$^{-1}$ (OH), 2920 cm.$^{-1}$ (C—H), 1600 cm.$^{-1}$ (trityl), 1085–1140 cm.$^{-1}$ (—C—O—C—).

Compound III was reacted with octadecyl bromide in the presence of powdered KOH in benzene (6), resulting in 1-*O*-octadecyl-2,2-dimethoxy-3-*O*-trityl propane (IV). IR spectrum showed loss of OH band absorption, 2920 cm.$^{-1}$ (C—H, substantial increase in aliphatic CH band absorption), 1085–1140 cm.$^{-1}$ (—C—O—C—). Hydrogenolysis of IV with 15% Pd/C in dioxane at 40° and 60 p.s.i. resulted in I.

Anal.—Calcd. for $C_{21}H_{42}O_3$: C, 73.63; H, 12.36. Found: C, 73.59; H, 12.29.

IR spectrum: 3420 cm.$^{-1}$ (OH), 2920 cm.$^{-1}$ (C—H), 1725 cm.$^{-1}$ (—C—C—C—), 1085–1140 cm.$^{-1}$ (—C—O—C—). NMR in $CDCl_3$ showed no methoxy singlet, and the assignments of fragments in the mass spectra were comparable to that of the keto-enol form of I, m.p. 87–89°.

In the second synthetic scheme, batyl alcohol was benzoylated in the presence of pyridine and the crude mono derivative was oxidized with dimethyl sulfoxide and dicyclohexylcarbodiimide in the presence of trifluoroacetic acid (7). This afforded 1-*O*-octadecyl-3-*O*-benzoyl-2-propanone, V.

Anal.—Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.26; H, 10.29, m.p. 52–53°.

Compound V was then ketalized to afford 1-*O*-octadecyl-2,2-dimethoxy-3-*O*-benzoylpropane, VI, which was further confirmed with IR. Compound VI was base hydrolyzed to give 1-*O*-octadecyl-2,2-dimethoxy-3-hydroxypropane (II).

Anal.—Calcd. for $C_{23}H_{48}O_4$: C, 71.08; H, 12.45. Found: C, 70.82; H, 12.33. IR spectrum showed 3470 cm.$^{-1}$ (OH), 2920 cm.$^{-1}$ (C—H), 1085–1140 cm.$^{-1}$ (—C—O—C—). NMR δ 3.25 [S, 6. (OCH_3)], m.p. 29–31°.

The two synthetic preparations of *O*-alkyldihydroxyacetone were reduced with $LiAlH_4$ to the corresponding *O*-alkylglycerol, and GLC of the isopropylidene derivatives demonstrated that they had the same retention time as the isopropylidene derivative of authentic batyl alcohol. Acid hydrolysis of the ketone-containing lipids produced octadecanol; periodate oxidation produced *O*-alkylglycolic acids, and they could be reduced with $LiAlH_4$ to *O*-alkylethyleneglycols. These reactions and the details for identifying the products have been described (4). Further work on these ether-linked ketolipids is in progress in our laboratories.

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