[CONTRIBUTION FROM THE MEDICINAL CHEMISTRY BRANCH, CHEMICAL CORPS MEDICAL LABORATORIES]

CHOLESTERYL ESTERS OF METHYLPHOSPHONIC ACID

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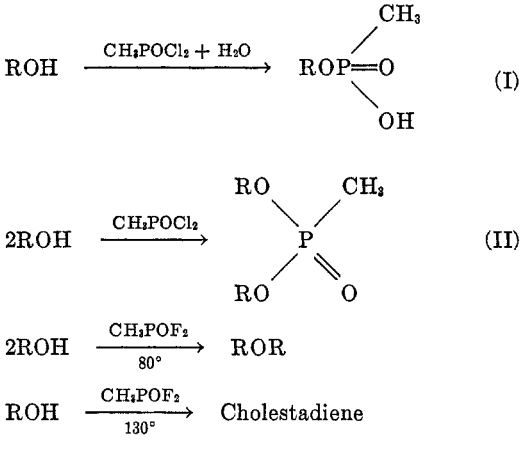
The molecular weight of the monocholesteryl ester of phosphoric acid, as determined in solution at temperatures below 100°, is considerably higher than the value calculated on the basis of a monomeric molecule (1). An assumption of dimerization appears to be confirmed by the demonstration that alkali metal salts may be obtained which contain only one atom of sodium or potassium for each two atoms of phosphorus (2). It has been assumed that association occurs by way of hydrogen bonds (3). To obtain more information on the mechanism of association of cholesteryl phosphates, it appeared desirable to prepare and study homologs of these compounds, in which one of the hydroxyl groups attached to the phosphorus has been replaced by a methyl group.

The reactions which have been carried out are shown in Table I.

TABLE I



R = Cholesteryl

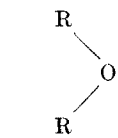
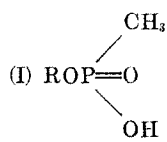
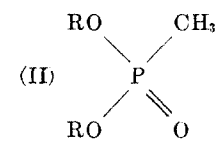
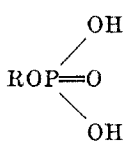
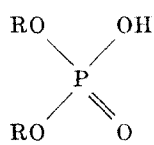
The monocholesteryl ester of methylphosphonic acid (I) can be obtained by interaction of excess methylphosphonic dichloride with cholesterol monohydrate in acetone-pyridine solution. This compound is soluble in aqueous potassium hydroxide solution, but not in sodium hydroxide solution, as was the case with cholesteryl phosphate. As was also the case with the latter compound, the molecular weight of the monocholesteryl ester of methylphosphonic acid in molten camphor was found to be 1.5 times the theoretical value.

The main reaction between 2 moles of cholesterol and 1 mole of methylphosphonyl dichloride in the absence of water yields the dicholesteryl ester of methylphosphonic acid (II) which shows no association in molten camphor. It

is an absolutely neutral molecule which, in contrast to dicholesteryl phosphate, gives no alkali salt, even when the hot solution in toluene is treated with the equivalent amount of sodium in ethanol. The presence of the methyl radical appears to diminish the tendency of the PO group to hydrate, so that no "*ortho* salt" (1) can be formed.

In Table II the melting points of cholesterol and its phosphoric and methylphosphonic esters are compared.

TABLE II

	M.P., °C.		M.P., °C.
ROH	146		195
	157–160		196–199
	175 (193)		204

R = Cholesteryl

One can see that esterification of one mole of cholesterol with methylphosphonic acid raises its melting point less than reaction with phosphoric acid itself. In the case of the dicholesteryl esters, the melting points are close to that of dicholesteryl ether; compound II also resembles this ether in its solubility properties.

The interaction of CH_3POF_2 on water-free cholesterol in boiling benzene gave dicholesteryl ether. At higher temperatures (in boiling xylene) more water is split off by the acylhalogenide to yield mainly cholestadiene (m.p. 73–76°, crystallized from acetone). Purification of the dark-colored reaction mixture was carried out by filtering through a layer of Al_2O_3.

EXPERIMENTAL

Cholesteryl ester of methylphosphonic acid (I) (*Methylcholesteroxyphosphonic acid*). To a mixture of 5.6 g. of methylphosphonic dichloride (CH_3POCl_2) and 28 ml. of dry acetone there was added, in portions and with cooling with running water, a solution of 5.6 g. of cholesterol (hydrate, recrystallized from 96% alcohol) in 28 ml. of water-free pyridine. After standing for 3 days at room temperature the reaction mixture was filtered through a fluted filter and the residue was washed once with water. The remaining material was dissolved in warm dilute potassium hydroxide solution and precipitated by acidifying with dilute sulfuric acid. Upon air-drying and recrystallization from boiling glacial acetic acid, it melted at 159–161°, and was soluble in ether, alcohol or *N*/2 KOH by moderate warming; insoluble in NaOH solution. The acid, when dissolved in ether and stirred into water, forms

a stable aqueous solution at *p*H 4–4.5, flocculating only when dilute mineral acid is added. For analysis the material was dried at 100° *in vacuo*.

Anal. Calc'd for $C_{28}H_{49}O_3P$: C, 72.5; H, 10.6; P, 6.7; M. W., 465.
Found: C, 72.2; H, 10.4; P, 6.7; M. W., 678, 715 (Rast, camphor).

Dicholesteryl ester of methylphosphonic acid (II). To a solution of 5 g. of anhydrous cholesterol (recrystallized from boiling benzene) there was added in portions (with frequent stirring) a mixture of 1.6 g. of methylphosphonic dichloride (CH_3POCl_2) and 20 ml. of dry acetone. On the following day the crystals were filtered off and were washed with dry acetone and ether. Upon crystallization from isopropyl alcohol containing a small amount of benzene, or preferably from glacial acetic acid, the material formed beautiful leaflets and melted at 196–198°. It was easily soluble in benzene or chloroform, rather insoluble in ether. For analysis the material was dried *in vacuo* at 100° over phosphorus pentoxide.

Anal. Calc'd for $C_{55}H_{93}O_3P$: C, 79.4; H, 11.25; P, 3.73; M. W., 833.4.
Found: C, 79.5; H, 11.2; P, 3.72; M. W., 810, 822 (Rast; camphor).

Acknowledgement. I wish to thank Mr. D. Levin for the molecular weight determinations.

SUMMARY

Methylphosphonic acid esters of cholesterol may be obtained under the proper conditions with either one or two molecules of the alcoholic component. The resulting esters are not very different in chemical characteristics from the corresponding esters of orthophosphoric acid. The apparent association shown by the monocholesterol ester of phosphoric acid can also be observed with the mono-ester of methylphosphonic acid, indicating that the replacement of one of the two hydroxyl groups attached to the phosphorus atom by a methyl group has not affected this particular characteristic. However, the dicholesteryl ester of methylphosphonic acid, which contains no free hydroxyl group, gives no evidence of association in melting camphor. These findings are in agreement with similar observations in other series of organophosphorus compounds in that only those substances which possess "true" acidic properties display association, whereas those substances lacking acidic properties are monomeric (4).

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REFERENCES

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