tioned. It is planned to study similarly the effects on respiration and salivation, as acetylcholine inhibits respiration and causes increased secretion of saliva. Other related humoral agents will also be investigated.

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A Suggested Biosynthesis of Cyclopropane Rings

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An unusual fatty acid was recently reported by Hofmann and Lucas (*1*). In addition to possessing an odd number of carbon atoms, the acid gives chemical reactions which point strongly to the presence of a three-membered ring. The authors formulated the structure in a general way as I.

$$CH_3(CH_2)_xCH—CH(CH_2)_yCOOH \quad (\text{CH bridging the two CH groups}) \qquad x + y = 14$$

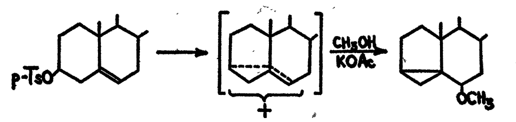
I

The x-ray diffraction pattern indicated a chain length in the 18-carbon range (*1*), an observation consistent with other information on the cyclopropane ring with regard to size and geometry (*2, 3*). Walsh (*2*), among others, has collected a good deal of information on the similarity of the cyclopropane ring to the ordinary double bond, focusing most attention on the availability of electrons (measured by spectra, conjugation, reactivity, etc.). Externally, the cyclopropane ring is analogous to a double bond, although "weaker" as an electron source, or as a conjugating group. Therefore, an organism which was not selective to a high degree would have a difficult time distinguishing between a C_{18} fatty acid and the C_{19} fatty acid containing the cyclopropane ring. The new acid was isolated from the lipid fraction of *Lactobacillus arabinosus*, and the acid may well serve to replace some C_{18} fatty acid.

Cyclopropane rings are a rarity in natural products, and only certain terpenes, the thujanes, caranes, and sabinenes, which contain a three-membered ring as well as a six, occur to any extent (*4*). Since three-membered rings are formed with relative difficulty in the laboratory, the biosynthesis of such rings becomes of interest. Most preparations utilize the displacement of halide by an anion formed by a strong base. Anion formation takes place when the hydrogens are "activated" by a neighboring group such as carbonyl which withdraws electrons. A typical synthesis (*5*) of this type is:

$$Cl—CH_2CH_2CH_2CN \xrightarrow{NaNH_2} [Cl—CH_2CH_2\ddot{C}H—CN] \longrightarrow CH_2—CH—CN \quad (\text{ring closed through } CH_2)$$

One known method of formation is more amenable to biosynthetic conditions. Stoll (*6*) discovered that solvolysis of cholesteryl *p*-toluenesulfonate in methanol buffered with potassium acetate led to the formation of an ether isomeric with cholesteryl methyl ether. Wallis *et al.* (*7*) in this country and Heilbron *et al.* (*8*) in England have elucidated the structure of the product, and Winstein (*9, 10*) has proposed a mechanism to fit the available data. The formation of isomeric (or i-) methyl ether may be formulated as follows:



The reaction is not limited to the sterol series, but no careful work on aliphatic compounds has been performed for the purpose of clarifying the mechanism.

The formation of the cyclopropane ring seems to depend on two related factors: (1) the ionization of a bond, and (2) the presence of a double bond in the proper relation to the ionizing bond. These are connected in the proposed mechanism because the double bond can aid the ionization by supplying electrons. Ionization also depends upon the character of the anion formed, and experience shows that the anions of strong acids (like the *p*-toluenesulfonate ion) are favorable.

With these facts in mind, a biosynthetic route to the acid, I, may be suggested. The precursor must possess the structure II in which *X* is a group which will promote the process of ionization. Ricinoleic acid, III, the only common (*11*) hydroxy-unsaturated fatty acid, fulfills the requirement after the hydroxyl has

$$R—\underset{X}{\overset{H}{C}}—CH_2CH{=}CHR'$$

II

$$CH_3(CH_2)_5\underset{}{\overset{O—H}{C}}HCH_2CH{=}CH(CH_2)_7COOH$$

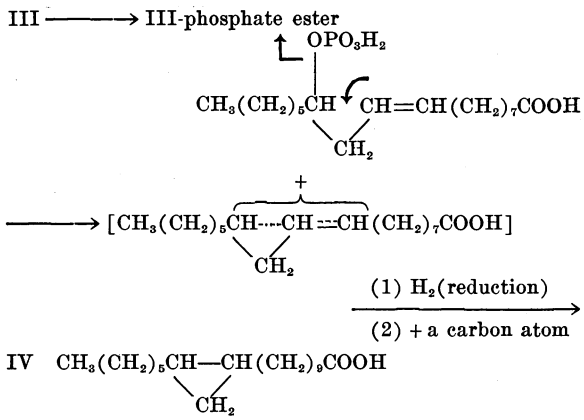
III

been converted to the phosphate ester by a phosphorylating enzyme system in a common variety of biochemical transformation. After or during ring closure, reduction would lead to a C_{18} acid; however, this acid would resemble a C_{17} straight chain acid and could conceivably be changed to a C_{19} acid (C_{18} analogue) by some such series of reactions as phosphorylation of the carboxyl group, condensation with acetate, conversion to a 2-ketoacid, and decarbonylation. On the foregoing basis, structure I will appear as IV. A chemical synthesis of IV could be designed with ricinoleic acid as starting material and utilizing the

[1] U. S. Public Health Service Research Fellow of the National Institutes of Health, 1949–52.

[2] The author would like to express his appreciation to S. Winstein for helpful discussion.

"i-sterol" effect for the formation of the cyclopropane ring in a manner parallel to the biosynthesis.



IV $CH_3(CH_2)_5CH(\diagdown CH_2\diagup)CH(CH_2)_9COOH$

Proof of this particular suggestion will depend on the validity of the structure IV proposed for the new fatty acid, but the general biosynthetic route outlined above may still be a reasonable hypothesis for the explanation of the formation of cyclopropane rings in natural products.

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Separation of Rusty Mottle of Cherry from a Ring-Spot–Rusty Mottle Complex[1]

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Most sweet and sour cherries carry the ring-spot virus in a more or less latent condition. This has caused considerable confusion in the study of cherry viruses on other species of *Prunus*. Often the symptoms described for the reaction on some specific host were not that of the original cherry virus but of the latent ring spot. In their work with mild rusty mottle virus, Zeller and Milbrath (*1*) reported the interference of the ring-spot virus in studying rusty mottle on peach. Whenever peach was inoculated with different sources of mild rusty mottle a reaction would occur which was typical of the ring-spot complex. At first this was believed to be a reaction of mild rusty mottle virus, since return inoculation from such peach trees always gave rusty mottle symptoms on cherry.

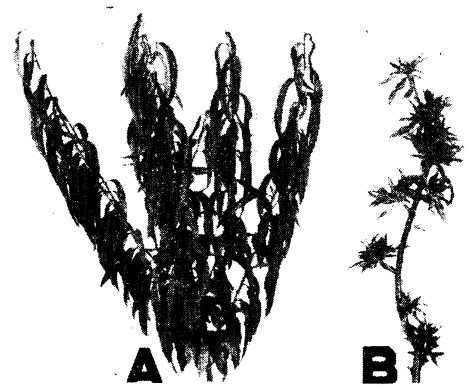

FIG. 1. Early Muir peach inoculated with: *A*, rusty mottle virus after removal of ring-spot complex; *B*, rusty mottle before removal of ring-spot complex.

From this work it became apparent that it would be valuable to have a method whereby the ring-spot virus could be separated from other stone fruit viruses. Since the ring-spot virus goes to most stone fruit varieties, separation on differential hosts based on susceptibility did not offer much promise. The local necrotic lesion type of reaction that occurs when ring-spot virus is budded into Shirofugen flowering cherry (*Prunus serrulata* var. Shirofugen) (*2, 3*) suggested a possible method of separation. If the ring-spot virus would move out more slowly than the second virus and remain somewhat localized in the inoculated branch, it would be possible to isolate the second virus beyond the region infected by ring spot.

In August three buds infected with ring spot and mild rusty mottle were placed near the terminal of a Shirofugen branch. By the following spring the inoculated branch showed severe gummosis and necrosis, indicating that the ring-spot virus had moved out into the Shirofugen. Buds were taken from several different branches not showing the ring-spot reaction and inoculated into Bing cherry. No symptoms of mild rusty mottle developed in the test tree, indicating the ring-spot virus had moved out of the infected bud, first killing a band of cells about the inoculation point and thus preventing the spread of the mild rusty mottle virus.

In order to avoid this direct exposure of Shirofugen tissue to the ring-spot virus before the mild rusty mottle virus had had an opportunity to move out into the Shirofugen, a second experiment was planned whereby both viruses would move through a non-necrotic host before coming in contact with Shirofugen. Ring-spot–free branches of Bing cherry were established on a 5-year-old Shirofugen tree. When these branches were 4–5 ft long, two cherry buds infected with both the ring-spot virus and mild rusty mottle virus were placed at the terminal portion of one of the

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