In a different area, the CI spectrum of free alanylvaline was studied. This spectrum differs quantitatively but not qualitatively from the EI spectrum (Figure 6). Both give a quasimolecular ion, and the important sequence-determining ion at *m/e* 72 $(CH_3CHNH_2CO)^+$ is present in both spectra, being the base peak in the CI spectrum. The intense ion at *m/e* 118 is presumably protonated valine $(C_5H_{12}NO_2)^+$.

Finally, the CI spectra of some steroidal ketones were studied. In this series, the formation of a quasimolecular ion at *m/e* (M + 1) as the base peak of the spectrum appears to be general, but the remarkable variation in the abundance of the peak corresponding to loss of water (0% in cholestan-3-one (Figure 7), 60% in androstan-17-one) suggests that location of the carbonyl group in the steroid nucleus may be determined in this way.

The foregoing examples are encouraging in that they confirm the original supposition that somewhat simpler fragmentations might prevail in CI mass spectrometry. The sensitivity of the spectrometer for important ions in the molecular ion region is increased by its being operated in CI mode, and these observations, taken together, permit the conclusion that the method merits further study, such as is being undertaken in these laboratories.

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A General Biochemical Synthesis of Oxygenated Prostaglandins E

Sir:

The prostaglandins are a family of compounds derived biosynthetically from unsaturated fatty acid precursors such as 8,11,14-eicosatrienoic or 5,8,11,14-eicosatetraenoic acids.[1,2] These substances have a widespread distribution in the body and exhibit a multitude of pharmacological effects.[3] Until now, no oxygenated prostaglandin E (PGE) has been synthesized or isolated from natural sources.[4] We herein describe a synthesis of 11α,15-(*S*)-dihydroxy-9,18-dioxo-5-*cis*,13-*trans*-prostadienoic acid (18-oxo-PGE_2), and 11α,15-(*S*)-dihydroxy-9,19-dioxo-5-*cis*,13-*trans*-prostadienoic acid (19-oxo-PGE_2). The principle of this method involves microbiological hydroxylation of 5,8,11,14-eicosatetraenoic acid; the resulting oxygenated unsaturated fatty acids or their derivatives can then be cyclized by exposure to bull seminal vesicle microsomes (BSVM) to yield the desired oxygenated prostadienoic acid derivatives.

Exposure of 5,8,11,14-eicosatetraenoic acid (I) (1 g) to *Ophiobolus graminis* afforded two polar products, which were characterized as 18ϵ-hydroxy-5,8,11,14-eicosatetraenoic acid (II) (200 mg) and 19ϵ-hydroxy-5,8,11,14-eicosatetraenoic acid (III) (170 mg) on the basis of the following data. Treatment of II with diazomethane afforded the methyl ester, whose mass spectrum showed a parent ion peak at *m/e* 334. Hydrogenation of the methyl ester over PtO_2, followed by chromic acid oxidation, resulted in the formation of the saturated keto ester, IV, mp 62°. Its mass spectrum exhibited the parent ion peak at *m/e* 340 with peaks at *m/e* 311 (M − 29) and *m/e* 269 (M − 71), corresponding to α and β cleavages as indicated. The methyl ester of III also showed a parent ion peak at *m/e* 334, consistent with the empirical formula, $C_{21}H_{34}O_3$. By a similar sequence of reactions, the saturated keto ester V, mp 56°, was obtained. Its nmr[5] spectrum exhibited bands at τ 8.72 (30 H, singlet, CH_2), 7.97 (3 H, singlet, $CH_3C{=}O$), 7.78 (4 H, multiplet, CH_2 adjacent to carbonyl), 6.4 (3 H, singlet, $-COOCH_3$). Its mass spectrum displayed a parent ion at *m/e* 340, and the characteristic peak[6] at *m/e* 283 (M − 57), corresponding to β cleavage as indicated, α cleavage of terminal methyl ketones being of minor significance.[6]

Although II and III could be cyclized to their corresponding prostadienoic acid derivatives by BSVM, the yields (<10%) were low. Furthermore, the 18- and 19-acetoxy or methoxy eicosatetraenoic acid derivatives were not cyclized by BSVM. On the other hand, both 18-oxo-5,8,11,14-eicosatetraenoic acid (VI) and 19-oxo-5,8,11,14-eicosatetraenoic acid (VII) obtained by Jones oxidation from II and III, respectively, were converted into their prostadienoic acid derivatives. In a typical experiment, VI (345 mg) was incubated with BSVM (14.3 g) in 0.05 *M* phosphate buffer, pH 7.5, in the presence of hydroquinone (207 mg) and glutathione (690 mg) for 5 hr at 37°. After the usual work-up,[7] followed by successive column chromatography on silicic acid, reverse-partition chromatography,[8] and silicic acid, 40 mg of 18-oxo-PGE_2 (VIII) was obtained. Alkaline treatment of VIII afforded a compound with absorption maxima at 278 mμ,[9] reminiscent of the prostaglandin B type chromophore, and at 330 mμ. Its nmr spectrum revealed signals at τ 8.96 (3 H, triplet, J = 3.5 cps), 8.26 (quartet, J = 3.5 cps), 5.88 (2 H, multiplet), 4.45 and 4.35 (6–7 H), characteristic of the E_2 type of prostaglandin.[10] The mass spectrum of VIII gave a parent ion at *m/e* 348 (M − 18) and *m/e* 330 (M − $2H_2O$), corresponding to the loss of the 11 and 15 hydroxyl groups; *m/e* 204 (330 − 126), a McLafferty cleavage at C-8 with loss of the carboxylic side chain; *m/e* 273 (330 − 57) and *m/e* 258 (330 − 72) represent α and β cleavages as indicated. 15-(*S*)-Hydroxy-9,18-dioxo-10-prostaenoic acid methyl ester (X) was prepared from VIII by methylation, hydrogenation over PtO_2,

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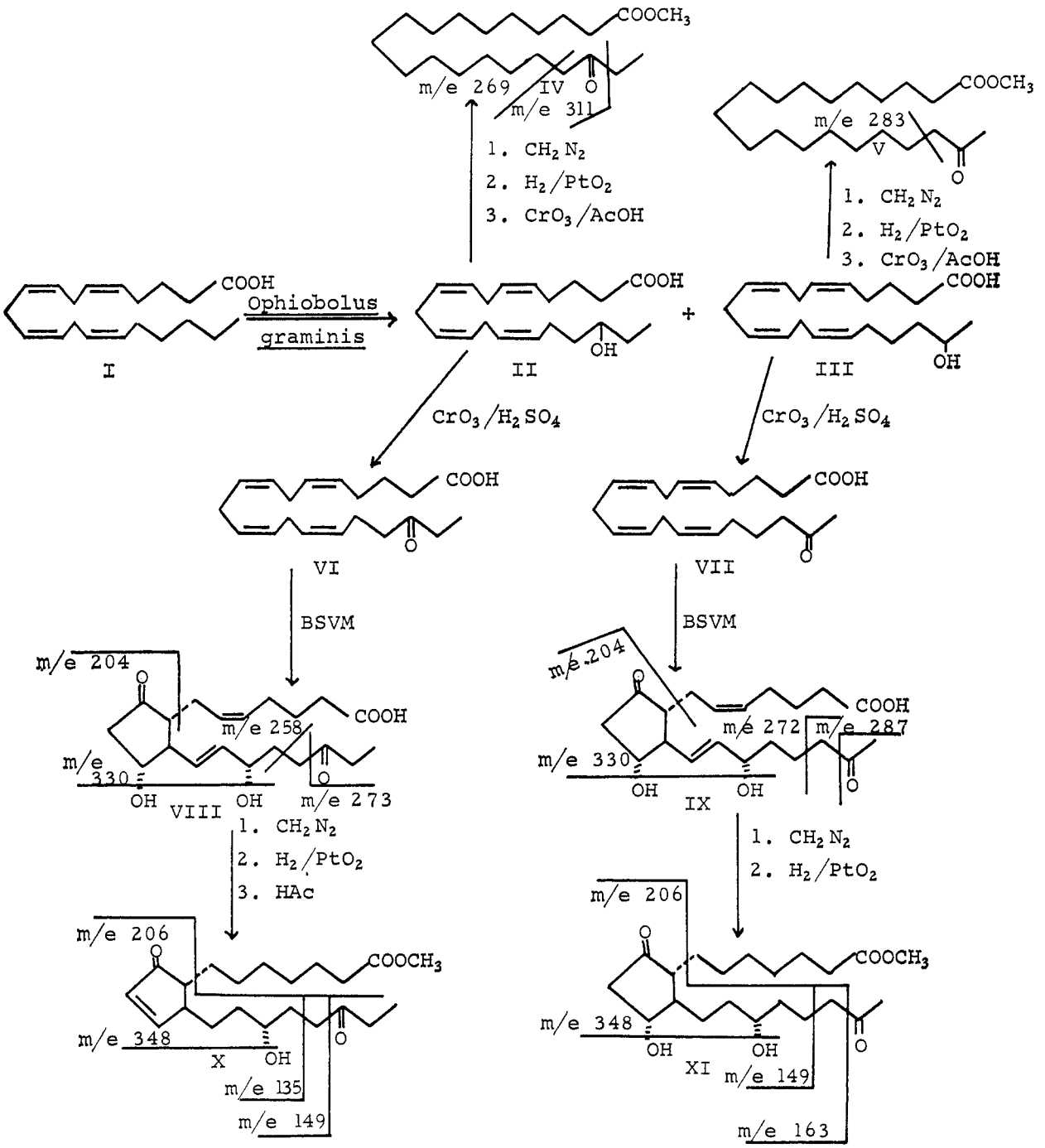

and dehydration with 90% acetic acid;[11] its ultraviolet spectrum showed a maximum at 222 mμ (ε 12,600); its mass spectrum gave a parent ion at *m/e* 366 with peaks at *m/e* 348 (M − 18), loss of the 15-hydroxyl group. The peak at *m/e* 206 corresponds to the loss of the carboxylic side chain *via* a McLafferty cleavage, followed by α and β cleavages to give *m/e* 149 and *m/e* 135, respectively.

Following the above procedure, VII (430 mg) was converted into 19-oxo-PGE_2 (IX) (48 mg) after exposure to BSVM. A compound with an absorption maximum at 278 mμ was also obtained upon alkaline treatment of IX. The mass spectrum of IX showed peaks at *m/e* 348 (M − 18), *m/e* 330 (M − $2H_2O$). The peaks at *m/e* 287 (330 − 43) and 272 (330 − 58) correspond to α and β cleavages and *m/e* 204 is in agreement with the loss of the carboxylic side chain. The nmr spectrum of IX exhibited signals at τ 4.14 and 4.32 (5 H), 4.62 (2 H), 5.9 (2 H, multiplet), 7.4–8.1 and 8.2–8.6 (multiplets) similar to prostaglandin E_2. In a similar fashion, 11α,15-(*S*)-dihydroxy-9,19-dioxoprostanoic acid methyl ester (XI) was obtained by methylation and hydrogenation of IX. Its mass spectrum showed peaks at *m/e* 366 (M − 18); α and β cleavages of *m/e* 206 at C-19 yielded *m/e* 163 and *m/e* 149, respectively. Dehydration of XI with 90% acetic acid gave a product with an ultraviolet absorption maximum at λ_{max} 221 mμ (ε 10,800).

The method herein described provides a relatively convenient route for the synthesis of oxygenated prostaglandin E derivatives, which would be exceedingly

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difficult to prepare by conventional partial or total chemical synthesis. It should now be possible to examine whether separation of biological activities could be accomplished by suitable oxygenation of the parent hormone.[12]

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The Mechanism of Formation of 1-Methylcyclobutanol from (β-Methylallyl)carbinylamine with Nitrous Acid

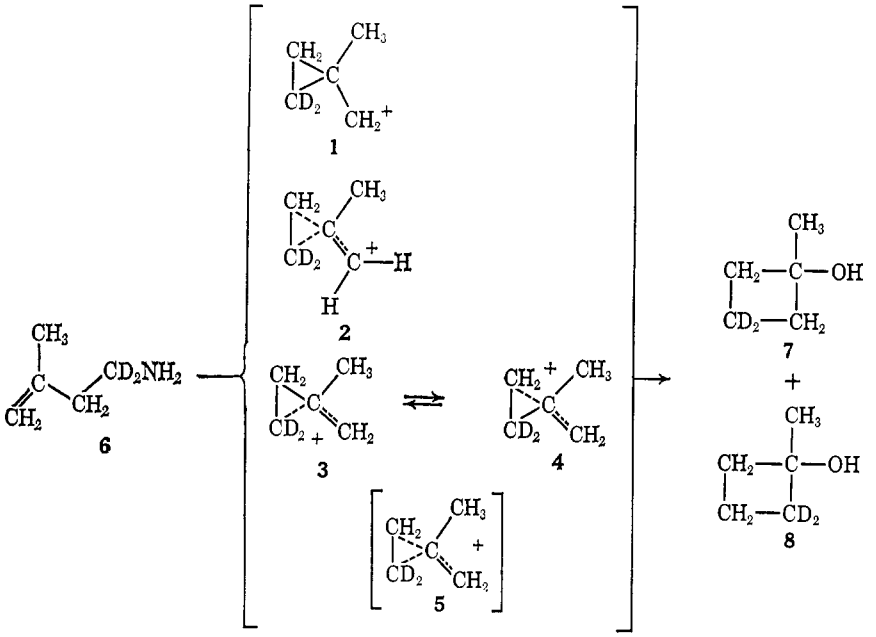
Sir:

Evidence has been presented for formation of a common intermediate(s) in the carbonium ion interconversion reactions of (1-methylcyclopropyl)carbinyl, (β-methylallyl)carbinyl, and 1-methylcyclobutyl derivatives.[2] The structure(s) of the intermediate(s) is unknown but, for the corresponding unsubstituted derivatives, it has been proposed that equilibrating delocalized "bicyclobutonium" cations might provide the most economical explanation.[3] However, the stereochemical properties of the intermediate(s) derived from methylcyclopropylcarbinyl derivatives are suggestive of a "symmetrical homoallylic" structure.[4,5] Indeed, except for the extensive formation of products with the cyclobutyl structure, equilibrating "homoallylic" cations can account for the methylene shuffling, anchimeric assistance, and product distributions observed in many of the carbonium ion reactions of these systems.[6] That the dimethylcyclopropylcarbinyl cation displays nmr signals indicative of nonequivalent methyl groups has led to the postulation of a "bisected" structure for the dimethylcyclopropylcarbinyl cation,[7,8] although the bicyclobutonium structure is not rigorously excluded by the nmr spectra. In general, it seems that the properties which might be ascribed to the "bisected" cation and the "symmetrical homoallylic" cation are not in fact really different.

A possible test for the importance of cations such as the classical (1-methylcyclopropyl)carbinyl cation (**1**), the "bisected cation (**2**), a rapid equilibrating mixture of "homoallylic" cations (**3** and **4**), or the "symmetrical homoallylic" cation (**5**) as intermediates in the interconversions of (1-methylcyclopropyl)carbinyl, 1-methylcyclobutyl, and (β-methylally)carbinyl derivatives is available by investigation of the deamination of 1,1-dideuterio-3-methyl-3-butenylamine (**6**). If any of these are, in fact, intermediates in the formation of 1-methylcyclobutanol, then this product would be an equimolal mixture of **7** and **8**.

The deuterated amine was synthesized from methyl 3-methyl-3-butenoate which was reduced to 1,1-dideuterio-3-methyl-3-buten-1-ol with lithium aluminum deuteride. The alcohol was converted to the benzenesulfonate with benzenesulfonyl chloride in collidine. The azide was then obtained with sodium azide in dimethyl sulfoxide and reduced to the amine with lithium aluminum hydride. The over-all yield was 34%, based on the lithium aluminum deuteride.

The deuterated amine was deaminated with sodium nitrite in 1 *N* aqueous perchloric acid. The resulting mixture of alcohols was separated by preparative vpc on four 12-ft Carbowax columns at 120° in a Beckman Megachrom. The impure 1-methylcyclobutanol was treated with bromine at 0° in carbon tetrachloride and



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