Oxidizing abilities of callus were already reported. For instance, pregnenolon was oxidized to progesterone by *Digitalis*, *Nicotiana* and *Sophora* callus.⁴⁾ However, it is the first time to be found in callus that those sterols having 4-ene-3-one and further oxidized 4-ene-3,6-dione groups were present together with normal phytosterols. Therefore, those fact is very interesting from the biogenetic point of view.

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A Novel Mannich Reaction Product from 2,4(5)-Diisopropylimidazole

The reactions of some imidazole derivatives with formaldehyde have been reported,^{1,2)} e.g. 1-methyl- or 1-benzylimidazole reacts forming the corresponding 2-hydroxymethylimidazole.³⁾ Stocker, *et al.*⁴⁾ reported that the Mannich reaction of imidazole derivatives occurs at unsubstituted positions of the imidazole ring.

With 2,4(5)-dialkylimidazoles (I),⁵⁾ the Mannich reaction should be possible at the 1 and/or 4(5) position. When 2-isopropyl-4(5)-*n*-propylimidazole (Ia: R¹=Pr^{iso}, R²=Prⁿ) was heated with formalin and dimethylamine in a sealed tube at 110–120°, various unstable products were formed and one of these, the Mannich reaction product, 2-isopropyl-4(5)-*n*-propyl-5(4)-dimethylaminomethyl imidazole (IIa: R¹=Pr^{iso}, R²=Prⁿ), was isolated by sublimation in poor yield.

The other products could not be isolated in a pure state. In the nuclear magnetic resonance (NMR) spectrum of IIa, the N-H signal was seen as a broad singlet centered at 8.27 ppm, which disappeared upon shaking with D₂O. The methylene proton signal of the

Chart 1

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dimethylaminomethyl group was seen at 3.18 ppm, and the N-methyl signal at 2.07 ppm. However, when 2,4(5)-diisopropylimidazole (Ib: $R^1=R^2=Pr^{iso}$) was treated in the same way, the corresponding dimethylaminomethyl derivative was not obtained, and another Mannich reaction product (IIIb), derived from two imidazole molecules and two formaldehyde molecules, was isolated in poor yield.

When Ib was heated with formalin, without added base in a sealed tube at 110–120° for 3 hr, IIIb was obtained in good yield (ca. 80%). The infra red spectrum of IIIb showed no NH absorption, the mass spectrum had a molecular ion peak at m/e 328, and the NMR spectrum had a methylene proton signal of the dihydropyrazine ring at 4.96 ppm (singlet). Strangely, Ia and other imidazoles ($R^1=CH_3$, $R^2=Pr^{iso}$; $R^1=Pr^{iso}$, $R^2=CH_3$; $R^1=Pr^{iso}$, $R^2=Et$) gave a trace of III under similar conditions and also under other conditions tested.

Formation of a similar ring system, but with two carbonyls instead of two methylenes, was reported by Godefroi, *et al.*⁶⁾ in the reaction of 4-methyl-5-imidazolecarboxylic acid with acetic anhydride. Oxidation of IIIb with potassium permanganate or chromic acid (Collins oxidation⁷⁾) gave many products which were not identified.

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Active Peptides on Smooth Muscle in the Skin of *Bombina orientalis* BOULENGER and Characterization of a New Bradykinin Analogue

We have examined some biological active peptides in the skin of korean frog, *Bombina orientalis* BOULENGER, and obtained a new bradykinin analogue in addition to bombesin and bradykinin. This communication deals with the separation and chemical characterization of these peptides.

Separation of Active Peptides

Skin of 5 frogs was extracted with 300 ml of methanol containing 3 ml of 6% trichloroacetic acid and the extract was evaporated in reduced pressure. After the removal of fatty material by extraction with 200 ml of *n*-hexane and 200 ml of ether, the residue was dissolved in 5 ml of 10% acetic acid and centrifuged. The supernatant was used for further purification. Separatory process of active peptides was summarized in Chart 1. Activity in each fraction was assayed by contraction of rat uterus,¹⁾ guinea pig ileum,²⁾ and guinea pig gall bladder³⁾ respectively. Smooth muscle contracting activity was found in three fractions (Bo-I, Bo-II, and Bo-III) in the first chromatography. In further purification of Bo-II, the active fraction

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