$C_5H_5VC_7H_8$, since the latter would be a cycloheptatriene derivative of vanadium[I] with two unpaired electrons. The vanadium compound should most probably be formulated as $C_7H_7^+V^0C_5H_5^-$(I). However, in the solid state it is stable in air at room temperature, only beginning to decompose at about 130°, in distinct contrast to the isoelectronic $(C_6H_6)_2V$.[6] Perhaps it is possible to explain the increased stability of $C_5H_5VC_7H_7$ over $(C_6H_6)_2V$ by regarding the former as a resonance hybrid of I and $C_7H_7^-V^{II}C_5H_5^-$(II). Although the cycloheptatriene anion, isoelectronic with a "planar" cycloöctatetraene molecule with eight π-electrons, is not normally stable, the existence of $C_8H_8Fe(CO)_3$ and $C_8H_8[Fe(CO)_3]_2$[7] indicates that stable sandwich compounds can be formed by 8 π-electron systems, suggesting that the canonical form (II) might have some importance.[8]

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[6] E. O. Fischer and H. P. Kögler, *Ber.*, **90**, 250 (1957).

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[8] Because of the possibility of occurrence of this type of resonance designation of the π-C_7H_7 group in $C_5H_5VC_7H_7$ as "cycloheptatrienyl" rather than "tropylium" seems preferable. According to conventions now in use it is also difficult, or even perhaps unwise, to assign a definite oxidation state to the vanadium.

[9] National Science Foundation predoctoral fellow 1958–1960.

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ISOLATION OF 5-METHOXYINDOLE-3-ACETIC ACID FROM BOVINE PINEAL GLANDS[1]

Sir:

During the isolation and characterization of melatonin,[2] a substituted 5-hydroxyindole derivative in the pineal gland that can lighten pigment cells, we found a chemically related but biologically inactive compound. The latter, now identified as 5-methoxyindole-3-acetic acid (I), is the first demonstration of O-methylation of a 5-hydroxyindole. This compound had not been known to exist in biologic tissue although it had been isolated as a urinary excretion product in rats after administration of 5-methoxytryptamine.[3]

One hundred grams of trimmed lyophilized bovine pineal glands[4] was powdered in a blender and defatted with petroleum ether in a soxhlet extracter. The powder was mixed with 1700 ml. water in a blender for two minutes and the mixture centrifuged at 16,000 × *g* for 30 minutes. The supernate was passed through glass wool and extracted twice with equal volumes of ethyl acetate. The ethyl acetate layers were taken to dryness *in vacuo*. The residue was subjected to countercurrent distribution of 29 transfers with the upper phase moving in a system of ethyl acetate:heptane:water in a 1:1:2 ratio. Tubes 7 to 22 were combined and taken to dryness *in vacuo*. The residue was dissolved in benzene and put on a silicic acid column. With 0.75% methanol in benzene, I was eluted, evaporated to dryness, and the residue rerun through the countercurrent distribution and the silicic acid column. The final product was sublimed at 75° for three hours *in vacuo* to give 400 μg. of I.

Ultraviolet light absorption maximum of I was at 2760 Å. with shoulders at 2960 and 3080 Å. No appreciable acid–base shift of the maximum occurred. Maximum fluorescence was at 338 mμ and maximum activation at 304 mμ. Infrared examination showed a broad carbonyl absorption at 1710 cm.$^{-1}$ and the possible presence of a methoxyl group. A strong blue violet color was obtained with Ehrlich reagent. Electrophoretic studies showed that the compound migrated as if it had a free carboxyl group.

Ultraviolet absorption and fluorescence properties and the reaction with Ehrlich reagent indicated that the compound was a 5-hydroxyindole derivative. The lack of a significant acid–base shift of the ultraviolet absorption maximum signified that the hydroxy group was blocked. The Ehrlich reaction indicated that positions 1 and 2 probably were free. Ultraviolet and infrared data showed that the carboxyl, detected by electrophoretic studies, was not conjugated with the ring.

Because extremely small amounts of material were available, final evidence for the structure of I was obtained by mass spectrometry. The methyl ester, prepared by treating I with diazomethane, had a mass of 219. A strong peak at mass 160 indicated the structure of a fragment as $CH_3O-C_8H_5N-CH_2-$. These data suggested that the structure for I was 5-methoxyindole-3-acetic acid. I was synthesized from 5-methoxyindole-3-acetonitrile.[5,6,7] The synthetic and natural compounds were found to have identical chemical and physical properties as determined by reaction with Ehrlich reagent; infrared, ultraviolet absorption and fluorescence curves; countercurrent distribution; silicic acid column; and paper chromatography with acidic, basic and neutral solvents. Mass spectrometry of the methyl esters of both synthetic and natural I revealed the same proportion of 219 and 160 masses.

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[1] This investigation was supported by grants from the American Cancer Society and the United States Public Health Service.

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[4] We are grateful to the Armour Laboratories for supplying us with many kilograms of bovine pineal glands.

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