midine fraction reached its maximum about 20 h after administration of 5 μC of putrescine. Incorporation into spermine was slower. After a lag period, the total activity seemed to increase up to at least 60 h. Of the total radioactivity injected into the egg, about 1 % could be detected in the polyamines 60 h after treatment.

Studies with labelled ornithine showed a similar incorporation. It was interesting to note that after paper electrophoretic separation radioactivity was also found in the "putrescine area". In control experiments it was demonstrated that this unknown compound (putrescine?) was not produced during the isolation procedure.

Incorporation of 2-^{14}C-methionine into spermidine was quite similar to that of putrescine (Fig. 1b). After administration of 5 μC of methionine the maximum of total counts was about 40 000 c.p.m. in spermidine at 30 h after injection. The incorporation of methionine into spermine was more rapid than that of putrescine. 60 h after injection, about 65 000 c.p.m. could be detected in spermine and 35 000 c.p.m. in the spermidine fraction. Of the total radioactivity injected into the egg about 2.8 % was recovered in the polyamines 60 h after administration.

The results described here demonstrate that putrescine, ornithine and methionine can act as precursors in the biosynthesis of spermine and spermidine in the chick embryo. In addition, spermidine seems to be a precursor of spermine.

A more detailed report of the methods and results described here will be published in the near future.

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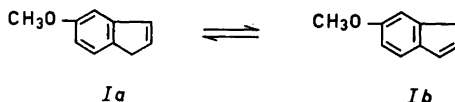
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On the Tautomerism of 5(7)H-1-Pyrindine
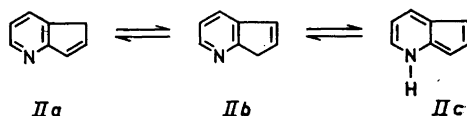
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In 1923, Ingold and Piggott [1] investigated the tautomerism of the three-carbon system of the methoxy-indenes Ia and Ib.

These two forms could not be separated, different synthetic methods only yielding identical samples. The single substance thus isolated gave, however, by oxidation a mixture of two methoxy-homophthalic acids, and on blocking the tautomeric system by condensation with piperonal, a mixture of two, separable, derivatives was obtained.
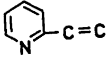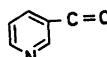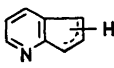
Robison [2] synthesized and investigated 5(7)H-1-pyrindine (II) in which the same kind of tautomerism is possible (IIa and IIb) as in the methoxyindenes. A third tautomeric form (IIc) with the proton attached to nitrogen is, however, also possible, and Anderson et al. [3] have suggested that this form is responsible for the

orange color of II as observed by Robison. Quite recently Reese [4] has confirmed this hypothesis in a very beautiful way, by synthesizing 1-methyl-1-pyrindine, discussed long ago by Armit and Robinson [5], and comparing its UV-spectrum with that of Robison's substance. The outcome of this investigation was that II contains 1.1 parts per 1000 of the coloured pseudoazulene IIc.

We have for some time been interested in the tautomerism of three-carbon systems, and in particular the tautomerism of 5(7)H-1-pyrindine and related compounds. Due to the current interest in this substance we wish to report the results

Talbe 1. Calculated and observed excitation energies.

Molecule	ΔE calc. (β_0)		ΔE calc. (kcal/mole)		ΔE obs. (kcal/mole)	
	ΔE_1	ΔE_2	ΔE_1	ΔE_2	ΔE_1	ΔE_2
(pyridine)—C=C	−1.308	−1.608	101.9	125.3	102.1	121.7
(pyridine)—C=C	−1.320	−1.566	—	122.0	102.88	120.2
(1H-1-pyrindine, N–H)	−0.987	−1.317	76.9	102.6	61.0	—
(pyrindine)—H					101.1	115.8

of a preliminary theoretical investigation of this problem. The molecules IIa and IIb are interesting since their σ-bond skeletons are equivalent and any difference in their properties should thus be due to differences in π-electron structure and energy. Furthermore, if hyperconjugation is neglected, the π-electron systems of IIa and IIb are the same as in 2- and 3-vinylpyridine, respectively, at least within the Hückel approximation. We have calculated the electronic excitation energies and the total π-electron energies of 2- and 3-vinylpyridine and 1H-1-pyrindine (IIc) by the standard Hückel-method neglecting overlap and with the parameters recommended by Pullman and Pullman [6].

The calculated and observed excitation energies are given in Table 1. The parameter β_0, occurring in the Hückel theory, was determined empirically from the first transition of 3-vinylpyridine, and the value thus obtained was used in the prediction of the other excitation energies. The first thing to be noticed is the prediction of a much lower excitation energy for IIc as compared to 2- or 3-vinylpyridine. The

numerical discrepancy between the calculated value and that observed by Reese is not surprising in view of the approximations involved in the theoretical method. In general, a good prediction is possible only within a series of related iso-π-electronic molecules. Furthermore, the agreement between the three other calculated and observed excitation energies is surprisingly good, and the difficulty of deciding between the two tautomers IIa and IIb from UV-spectra [2,4] is further substantiated by this theoretical result.

It is also interesting to note that the total π-electron energies of 2- and 3-vinylpyridine are identical (10.865 β_0) despite the small but significant differences in charge distribution and bond-orders. When IIa or IIb is exposed to conditions catalyzing a tautomeric rearrangement, one would therefore expect to obtain a mixture, and Robison's [2] remark: "it is likely that the material is a mixture of labile tautomeric forms", is certainly appropriate. The close energetic similarity between IIa and IIb is not unexpected if one remembers that both forms are derived from the same alternating hydrocarbon (indene), and this

point is the subject of our further investigations.

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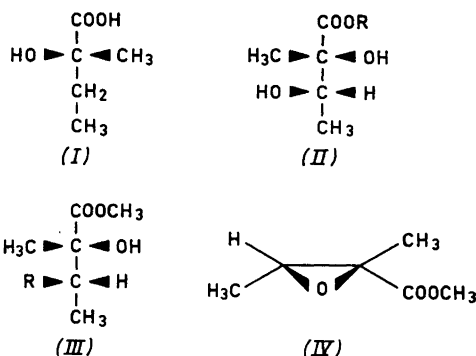## The Absolute Configuration of 2-Hydroxy-2-Methylbutyric Acid

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The absolute configuration is unknown of the simplest, asymmetric, tertiary α-hydroxy-acid, 2-hydroxy-2-methylbutyric acid (2-ethyl-lactic acid), occurring in ester linkage in the *Veratrum* alkaloids protoveratrine A [1,2] (protoveratrine [3,4]), deacetylprotoveratrine A [5] (deacetylprotoveratrine [6]), germerine [7,8] and germitrine [8], as well as in the bitter principle glaucarubin [9]. We now report that the naturally derived *dextro*rotatory acid possesses the (S)-configuration (I).

Upon reaction with diazomethane, (+)-*threo*-2,3-dihydroxy-2-methylbutyric acid, to which we recently assigned the (2R,3S)-configuration (II,R=H) [10], afforded the corresponding methyl ester (II, R=CH₃)*, b.p. 45°/0.1 mm, n_D^{25} 1.4428, $d_4^{21.5}$ 1.141, $[\alpha]_D^{22} - 2.5°$ (neat), which was further converted into the *p*-toluenesulphonate (III, R=*p*-CH₃C₆H₄SO₂O), m.p. 66−67°, $[\alpha]_D^{23}$

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* Satisfactory analyses and consistent infrared and NMR-spectra have been obtained for all new compounds reported.

(I), (II), (III), (IV)

−6.0° ((*c* 11.5, CHCl₃). Treatment of the latter with sodium methoxide in methanol yielded a glycidic ester, b.p. 54°/11 mm, n_D^{25} 1.4160, $d_4^{21.5}$ 1.042, $[\alpha]_D^{22} + 21.1°$ (neat), possessing the configuration (IV) on basis of the well-established inversion accompanying ring-closure reactions of the present type, induced through nucleophilic displacement. Nucleophilic attack on (IV) with methyl mercaptide, again accompanied by inversion at C-3, afforded the sulphide (III, R=CH₃S), b.p. 100°/10 mm, n_D^{25} 1.4737, d_4^{22} 1.107, $[\alpha]_D^{25} - 6.7°$ (neat). Raney nickel hydrogenolysis converted this sulphide into the methyl ester (III, R=H), b.p. 153°, n_D^{25} 1.4137, d_4^{21} 1.002, $[\alpha]_D^{25} - 6.45°$ (neat). Lastly, alkaline hydrolysis of the ester yielded (R)-2-hydroxy-2-methylbutyric acid, m.p. 73.5−74.5°, $[\alpha]_D^{25} - 1.5°$ ± 0.1° (*c* 1.7, H₂O); −6.9° (*c* 1.5, 0.2 N NaOH); −8,5° (*c* 3.0, CHCl₃), *p*-phenylphenacyl ester, m.p. 122°, $[\alpha]_D^{22} - 2.1°$ (*c* 3.0, CHCl₃). The melting points agree with literature values [2,6-9] whilst the optical rotations of the acid are equal in magnitude * but *opposite in sign* to those reported for the *dextro*rotatory acid of natural

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* Except for one set of rotation values [8] ($[\alpha]_D^{24} + 4.4°$(*c* 1.6, H₂O); + 12° (*c* 1.28, 0.2 N NaOH)) which we consider to be somewhat too high. (The original preparation has deteriorated and redeterminations of the reported rotations hence are excluded. Slightly too high polarimeter readings seem to be the only possible source of error (Dr. O. Wintersteiner, private communication)).