SPECIAL ARTICLES

A FURTHER NOTE ON THE IDENTITY OF VITAMIN H WITH BIOTIN[1, 2]

IN a previous communication[3] we reported that a preparation of crystalline biotin methyl ester possessed vitamin H activity to a very high degree. This observation provided additional and direct support for our earlier conclusion[4] that vitamin H and biotin are "either identical or indeed closely related compounds." The sample employed in the investigation[3] was a solution of 150 γ of crystalline biotin methyl ester in ethyl alcohol that had been kindly furnished us by Professor F. Kögl. This material, which had been isolated from egg yolk, was shown by our assays with the rat curative method to contain 10,000 vitamin H units per mg. We now wish to report confirmation of the vitamin H activity of biotin by assays of crystalline biotin methyl ester isolated by us from a liver concentrate.

The first crystalline product we obtained from the liver concentrate possessed the same crystalline form as reported by Kögl and Tönnis[5] but showed a slightly higher melting point of 154° in contrast to that of 148° reported by them. It also showed a somewhat higher biotin activity (about 20 per cent.) when assayed against the sample furnished us by Kögl. The vitamin H assays, likewise, were proportionately higher. In view of this somewhat higher melting point and activity, we became interested in determining whether further purification were possible.

By repeated crystallization of the compound from a mixture of methanol and ether, a product of constant melting point and biotin activity was obtained which melted sharply in the Kofler micro-melting point apparatus at 166–167° (uncorrected), a melting point 18–19° higher than that reported by Kögl and Tönnis. The compound crystallized in long thin plate-like crystals from the methanol-ether mixture but crystallized in needles from chloroform by the addition of petroleum ether as described by Kögl and Tönnis. A full presentation of the chemical and physical properties of the compound will appear elsewhere. The biological activity and melting point of the pure biotin methyl ester were confirmed by several separate isolations. Furthermore, sublimation *in vacuo* followed by crystallization from a mixture of methanol and ether did not change the melting point, crystalline form or biological activity.

Using the yeast growth method,[6] we have compared the biotin activity of our purified crystalline material with the biotin activity of the vitamin H preparation (34 vitamin H units per mg) that we had used as an arbitrary standard in our previous communication.[3] *Expressed in terms of vitamin H units,* the various preparations of purified product that we have made have all consistently yielded, in the yeast growth method, the high value of 27,000 (± 10 per cent.) vitamin H units per mg. Direct vitamin H assays of our crystals, made on rats by the curative method, are in agreement with this high potency.

It may be added that Professor Dean Burk, using *Rhizobium trifolii* Strain 209, has found that our crystals possess "coenzyme R activity" of maximum observed potency, half-maximum growth being obtained at about 0.00001 γ per cc of growth medium and 95–100 per cent. maximum growth at 0.0001 γ per cc. This confirms, with strictly pure biotin ester, the conclusion of Nilsson, Bjälfve and Burström,[7] and of West and Wilson[8] that coenzyme R and biotin are identical. Our crystalline biotin ester was also found to be effective in promoting the growth of *Clostridium butylicum* Strain 21, by Dr. D. W. Woolley, of the Rockefeller Institute for Medical Research. Dr. Woolley's quantitative comparison of this crystalline material with Kögl's sample, made by assay with this organism, confirmed the ratio of potencies given above.

In conclusion we may state that the crystalline biotin ester prepared by us, purified to constant melting point and constant biotin activity, possesses such an extremely potent vitamin H activity—far beyond any previously known degree of activity—as to leave no doubt that vitamin H and biotin are one and the same compound. The behavior of the material in our hands has convinced us that we are dealing with an individual compound possessing the several biological activities mentioned in this note.

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[1] In this cooperative research reported here, the Cornell group has been responsible for the chemical work and for the biotin assays, and the Western Reserve group for the vitamin H assays.

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