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The Absolute Configuration of Biotin[*]

James Trotter and Jean A. Hamilton

ABSTRACT: The structure and the relative configuration at each of the asymmetric centers of biotin have been determined previously by an X-ray crystallographic analysis of the bis-*p*-bromoanilide of CO_2-biotin. By studying the anomalous dispersion of X-rays by crystals of this derivative, the absolute stereochemistry of biotin has now been established.

The structure of the bis-*p*-bromoanilide of carbon dioxide biotin has been determined by X-ray crystallographic analysis (Bonnemere *et al.*, 1965), and, as is usual in this type of analysis, only the relative configuration at each of the asymmetric centers of biotin was obtained. The results are in good agreement with those obtained from an X-ray study of unsubstituted biotin, where again only the relative configurations were determined (Traub, 1956, and personal communication, 1964). It is, however, possible, by careful measurement of the anomalous dispersion of X-rays (Bijvoet *et al.*, 1951), to determine the absolute configuration, and the present paper describes a series of such measurements for the bis-*p*-bromoanilide of CO_2-biotin, which establishes the absolute stereochemistry of biotin.

Structure factors were calculated for all the *hkl* the $\bar{h}\bar{k}\bar{l}$ reflections of the CO_2-biotin derivative from the final positional and isotropic thermal parameters (Bonnemere *et al.*, 1965, Table I). The scattering factors used for the two bromine atoms and for the sulfur atom were of the form

$$f = (f_o + \Delta f') + i\Delta f''$$

with $\Delta f'$, $\Delta f''$ for Cu Kα radiation (International Tables for X-Ray Crystallography, 1962). Many of the reflexions showed significant differences between $|F_c(hkl)|$ and $|F_c(\bar{h}\bar{k}\bar{l})|$, and several of the planes with the largest indicated differences were chosen at random and the intensities were measured on a General Electric XRD-5 spectrogoniometer. This instrument gives more accurate measurements and permits the detection of small intensity differences with more reliability than the photographic recording used in the original X-ray analysis. It would be possible to remeasure all the intensities and refine the structure further but, since the analysis of Bonnemere *et al.* was considered to be a sufficiently detailed one, only a few reflections were measured to deduce the absolute configuration. For these measurements a single crystal was chosen from the same sample that was used in the original X-ray analysis, the crystal was carefully aligned on the goniostat, and the various instrumental settings were precomputed from the measured cell dimensions (Bonnemere *et al.*, 1965). The integrated intensities were then measured with a θ–2θ scan, using a scintillation counter and approximately monochromatic Cu Kα radiation (nickel filter and pulse height analyser), and corrections for background counts were made.

The results of these measurements are given in Table I, I_o being measured intensities and $|F_c|$ calculated structure amplitudes. The results indicate unambiguously that $I_o(hkl)/I_o(\bar{h}\bar{k}\bar{l}) \approx |F_c(\bar{h}\bar{k}\bar{l})|^2/|F_c(hkl)|^2$, and hence the true absolute configuration is the optical enantiomorph of that shown in all the diagrams in the paper by Bonnemere *et al.* The correct configuration is obtained by referring the positional parameters of Bonnemere *et al.* (Table I of their paper) to a *left-*

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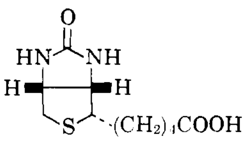
TABLE I: Determination of the Absolute Configuration of the Bis-*p*-bromoanilide of CO_2-Biotin (Cu Kα Radiation).

h	k	l	$I_o(hkl)$	$I_o(\bar{h}\bar{k}\bar{l})$	$\lvert F_c(hkl)\rvert$	$\lvert F_c(\bar{h}\bar{k}\bar{l})\rvert$	$\frac{I_o(hkl)}{I_o(\bar{h}\bar{k}\bar{l})}$	$\frac{\lvert F_c(\bar{h}\bar{k}\bar{l})\rvert^2}{\lvert F_c(hkl)\rvert^2}$
2	1	1	4925	7325	37.2	32.9	0.67	0.78
1	2	1	8975	7815	49.4	57.1	1.15	1.16
1	3	1	6380	9146	48.5	44.1	0.70	0.83
11	3	1	856	743	47.5	51.3	1.15	1.16
10	4	1	875	1125	33.3	29.2	0.78	0.77
6	6	1	791	618	20.4	25.8	1.28	1.60
6	1	2	4460	5176	54.1	50.2	0.86	0.86
6	2	2	145	111	14.1	21.2	1.32	2.26
5	3	2	1700	1670	37.8	42.5	1.02	1.26
8	3	2	1325	1193	36.8	42.3	1.11	1.32
2	10	2	836	1045	46.4	39.8	0.80	0.74
8	2	3	1305	1046	50.7	56.4	1.25	1.23
5	5	3	470	371	22.9	27.2	1.27	1.42
1	6	4	1260	1325	51.4	48.3	0.95	0.88

handed set of axes. The absolute stereochemistry of biotin is therefore given by structure I.



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