508. *The Circular Dichroism of N-Thiobenzoyl-L-α-amino-acids in Solution in Ether and in Methanol*

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Ethereal solutions of (ten) *N*-thiobenzoyl-L-α-amino-acids show negative circular dichroism centred at ~390 mμ. The shift of peak centre to ~386 mμ, observed with methanol solutions, is accompanied by inversion of sign of the circular dichroism with six of the compounds. A common spatial relationship between chromophore and asymmetric centre is thus adopted by these compounds in solution in ether, but not in methanol.

Related studies of *N*-substituted L-α-amino-acids, which were intended to demonstrate an empirical correlation between the sign of the Cotton effect or of the circular dichroism with absolute configuration, have uncovered apparent anomalies. The present work suggests that these also could be brought into line by the use of an appropriate solvent for each series.

Introduction of substituents linked by a thiocarbonyl group to the amino-group of D- and L-α-amino-acids yields derivatives showing anomalous optical rotatory dispersion (ORD) and, implicitly, circular dichroism (CD) in the accessible wavelength regions.[1-4] Series of such derivatives which have been prepared for ORD and CD studies are listed in Table 1. Xanthates derived from D- and L-α-hydroxy-acids [1,4] and alcohols,[4,5] and

Table 1

Anisotropic absorption maxima of *N*-substituted amino-acids in methanol

Substituent	$\lambda_{max.}$ (mμ)	log ε	Technique and ref.
RS·CS	333	2·0	ORD,[1,2] CD [4]
EtO·CS	285	2·2	ORD,[2] CD [4]
Ph·CH_2·CS *	330	1·8	ORD [3]
Ph·CS *	365	2·4	ORD [3]
HN·CS·NPh·CO·CHR	310	2·2	ORD,[2] CD [4]

CD measurements in methanol and/or dioxan

* Cyclohexylammonium salt.

N-acylthioureas [4,6,7] derived from carboxylic acids in which the α-carbon atom is asymmetric, similarly show accessible anomalous ORD and CD.

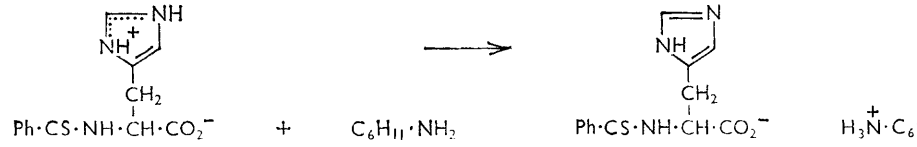
ORD studies with dithiocarbamates and xanthates derived from L-α-amino- and -hydroxy-acids led to the proposal of an empirical rule correlating the sign of the Cotton effect with absolute configuration in these series.[1] Phenylthiohydantoins derived from L-amino-acids were later [2,4] shown to conform to this rule, but apparent exceptions arose in related studies with a series of *N*-(ethoxythiocarbonyl)-L-amino-acids [2] and with the cyclohexylammonium salts of a series of *N*-(phenylthioacetyl)- and *N*-thiobenzoyl-D- and -L-amino-acids.[3]

At first unaware of the earlier study,[3] we prepared the *N*-thiobenzoyl derivatives of twelve α-amino-acids (D- and L-isomers of two, and L-isomers of the other ten amino-acids were used) and measured the ultraviolet spectra and circular dichroism of ten of these compounds in ethereal solution (the derivatives of L-alanine, L-aspartic acid, D- and L-glutamic acid, L-leucine, hydroxy-L-proline, L-phenylalanine, L-proline, D- and L-serine, L-tyrosine, and L-valine were soluble in ether, whereas the derivatives of L-histidine and of

[1] B. Sjöberg, A. Fredga, and C. Djerassi, *J. Amer. Chem. Soc.*, 1959, **81**, 5002.
[2] C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry, and B. Sjöberg, *Acta Chem. Scand.*, 1951, **15**, 903.
[3] B. Sjöberg, B. Karlen, and R. Dahlbom, *Acta Chem. Scand.*, 1962, **16**, 1071.
[4] C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Amer. Chem. Soc.*, 1962, **84**, 4552.
[5] B. Sjöberg, D. J. Cram, L. Wolf, and C. Djerassi, *Acta Chem. Scand.*, 1962, **16**, 1079.
[6] C. Djerassi and K. Undheim, *J. Amer. Chem. Soc.*, 1960, **82**, 5755.
[7] C. Djerassi, K. Undheim, and A.-M. Weidler, *Acta Chem. Scand.*, 1962, **16**, 1147.

L-lysine were insoluble in this solvent). The solutions showed circular dichroism associated with the low-intensity (log ε ~2·3) absorption maximum centred at ~395 mμ in their ultraviolet spectra. All the solutions of *N*-thiobenzoyl-L-amino-acids showed negative circular dichroism,* and the two corresponding D-isomers showed positive circular dichroism. It was therefore concluded that *N*-thiobenzoyl derivatives of α-amino-acids are suitable for the spectroscopic assignment of absolute configuration to this class of compound, although it was then seen that Sjöberg, Karlen, and Dahlbom [3] had reached the opposite conclusion. These workers determined the ultraviolet spectra and ORD of methanol solutions of the cyclohexylammonium salts of five *N*-thiobenzoylamino-acids; the low-extinction band was located near 365 mμ (log ε ~2·4) in this solvent, in which the compounds derived from L-glutamic acid, L-leucine, L-asparagine, and D-phenylglycine showed positive Cotton effects, and that derived from L-proline showed a negative Cotton effect.

In view of the different conclusions from the two investigations, we measured the ultraviolet spectra and circular dichroism of methanol solutions of those *N*-thiobenzoylamino-acids prepared for the present study, and of their cyclohexylammonium salts (*N*-thiobenzoyl-L-lysine was insoluble in methanol and did not form a cyclohexylammonium salt). The low-intensity absorption of the acids was located as a distinct maximum within the range 374—378 mμ, and that of the salts as an inflection at 369—370 mμ (log ε ~2·3); the ultraviolet spectra of thiobenzamide, in ether and in methanol, revealed a similar solvent shift in the position of the low-intensity (log ε 2·38) absorption ($\lambda_{max.}$ 404 mμ in ether; $\lambda_{infl.}$ 380 mμ in methanol, with no displacement on addition of cyclohexylamine). A definite correlation of the sign of the circular dichroism with absolute configuration was not established for methanol solutions; the *N*-thiobenzoyl derivatives of L-alanine, L-histidine, hydroxy-L-proline, L-proline, and L-serine showed negative circular dichroism, whilst the derivatives of the other six L-amino-acids showed positive circular dichroism. Each cyclohexylammonium salt, except that of *N*-thiobenzoyl-L-histidine, adopted the sign of circular dichroism of its parent *N*-thiobenzoylamino-acid. The results reported by Sjöberg, Karlen, and Dahlbom [3] are thus directly confirmed for the derivatives of L-glutamic acid, L-leucine, and L-proline, and their implications are given additional support through corresponding results obtained with several other derivatives. There is also eliminated a possible reason (*i.e.*, a consequence of the use of the cyclohexylammonium salts for ORD measurements) for the lack of correlation in this particular series; inversion of sign of circular dichroism through the conversion of *N*-thiobenzoyl-L-histidine into its cyclohexylammonium salt may be ascribed to a gross change of electronic environment at the asymmetric centre due to the removal, through salt formation, of a proton from the imidazolium ion in the side-chain:

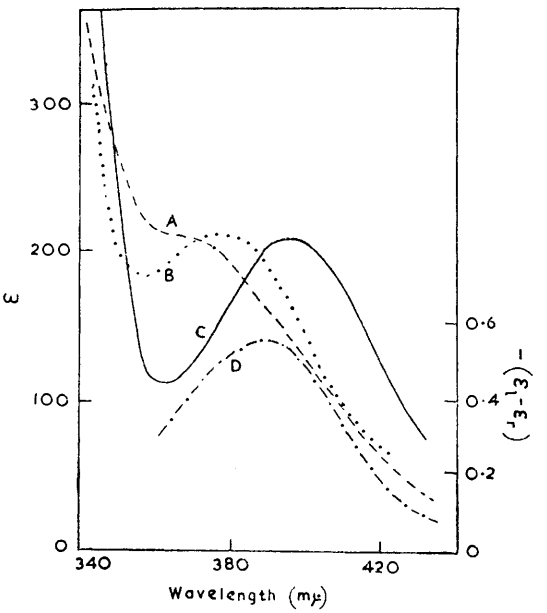


A change in the electronic environment at the asymmetric centre must also result from removal of the carboxyl proton during the conversion of *N*-thiobenzoylamino-acids with non-basic side-chains into their cyclohexylammonium salts; this change evidently affects $(\varepsilon_l - \varepsilon_r)_{max.}$ values, but not the sign, of the circular dichroism of the series.

* The $(\varepsilon_l - \varepsilon_r)_{max.}$ values determined for ethereal solutions of *N*-thiobenzoyl-L-phenylalanine and of *N*-thiobenzoyl-L-tyrosine with, however, close to zero; ill-defined Cotton effects, relative to those shown by corresponding derivatives of α-amino-acids carrying aliphatic side-chains, have been reported for the phenylthiohydantoins [2,4] (in methanol) and for the *N*-phthaloyl-compounds [8] (in methanol) derived from these amino-acids. Similarly, *N*-(ethoxythiocarbonyl)phenylalanine (in dioxan) yields [4] an ORD curve which rises through an inflection to large positive rotations at the shorter wavelengths.

[8] H. Wolf, E. Bunnenberg, and C. Djerassi, *Chem. Ber.*, 1964, **97**, 533.

Several *N*-thiobenzoyl-L-amino-acids thus do not conform to Sjöberg, Fredga, and Djerassi's rule [1] when methanol is used as solvent, though the ether-soluble derivatives are now shown not to be inherently exceptional. The discovery that the sign of circular dichroism of certain *N*-thiobenzoyl-L-amino-acids is inverted in methanol relative to that in ether implies [9] their adoption of a distinctly different spatial relationship between chromophore and asymmetric centre in the two solvents. Those derivatives (of L-alanine, hydroxy-L-proline, L-proline, and L-serine) which, in common with the other members of the series studied in ethereal solution, show negative circular dichroism, but which differ from the other members of the series in retaining this sign in methanol, evidently do not, or, possibly for structural reasons, cannot adopt in methanol the conformation preferred by the *N*-thiobenzoyl derivatives of other amino-acids. Similar departures (derivatives of



Ultraviolet absorption spectra of (A), cyclohexylammonium *N*-thiobenzoyl-L-glutamate (in MeOH); (B), *N*-thiobenzoyl-L-glutamic acid (in MeOH); and (C), *N*-thiobenzoyl-L-glutamic acid (in ether). (D) Circular dichroism of *N*-thiobenzoyl-L-glutamic acid (in ether)

histidine and serine have not been included in related investigations) from empirical correlations of sign of Cotton effect, or of circular dichroism, for series of *N*-substituted amino-acids with their absolute configurations involve derivatives of the prolines; namely, the *N*-(ethoxythiocarbonyl) derivatives [2] of hydroxy-L-proline and of acetoxy-L-proline, and the cyclohexylammonium salts of the *N*-(phenylthioacetyl) derivatives [3] of L-proline and of hydroxy-L-proline. The present results suggest that these departures might be brought into line by the use of an appropriate solvent for ORD or CD measurements in these series.

The use of *N*-thiobenzoyl derivatives for the spectroscopic assignment of absolute configuration to α-amino-acids carries certain practical advantages. Especially in ethereal solution, the " optically active " absorption maximum is found at a longer wavelength than with other *N*-derivatives whose ORD or CD has been investigated (see Table 1), and is well separated from the more intense absorption at shorter wavelengths. Also, the derivatives are available in high yield by a procedure which can be conveniently downscaled (5 mg. L-alanine yielded enough of its *N*-thiobenzoyl derivative to allow column-chromatographic purification, and enough crude *N*-thiobenzoyl-L-alanine was obtained from 1 mg. of the amino-acid to show distinct circular dichroism in ethereal solution). The derivatives

[9] C. Djerassi and L. E. Geller, *Tetrahedron*, 1958, **3**, 319; C. Djerassi, L. E. Geller, and E. J. Eisenbraun, *J. Org. Chem.*, 1960, **25**, 1.

are prepared [10,11] by aminolysis of *S*-thiobenzoylmercaptoacetic acid in neutral aqueous solution:

$$Ph{\cdot}CS{\cdot}S{\cdot}CH_2{\cdot}CO_2^- + NH_2{\cdot}CHR{\cdot}CO_2^- \longrightarrow Ph{\cdot}CS{\cdot}NH{\cdot}CHR{\cdot}CO_2^- + HS{\cdot}CH_2{\cdot}CO_2^-$$

Reaction with certain amino-acids is essentially complete at room temperature in less than ½ hr., but generally a period of 4—12 hr. is required for optimum yields. Reaction rates are increased at 50—60°, without diminution in overall yield, with, for example, glycine and L-alanine, though considerable racemisation occurred when the reaction with L-alanine was accelerated in this way. The procedure is, essentially, Holmberg's original method [10] which has been shown to involve no racemisation through the conversion of *N*-thiobenzoyl-L-amino-acids and *N*-thiobenzoyl-L-amino-acid amides prepared by the method into the corresponding *N*-benzoyl-L-amino-acids (through the use of alkaline hydrogen peroxide [10]) and *N*-benzoyl-L-amino-acid nitriles (through the use of mercuric acetate [11]), respectively. The products showed optical rotations close to those of the compounds prepared by Schotten–Baumann benzoylation. The conversion of *N*-thiobenzoylamino-acids into their benzoyl analogues is now found to be conveniently achieved by treatment, in acetone solution, with two equivalents of aqueous silver nitrate.[12]

Experimental

Ultraviolet spectra and CD measurements were determined using an Optica double-beam grating spectrophotometer and a Roussel-Jouan Dichrographe, respectively.

Preparation of N-*Thiobenzoylamino-acids.*—*N*-Thiobenzoylglycine and *N*-thiobenzoyl-D- and -L-glutamic acids were prepared by Holmberg's procedure.[10] All other *N*-thiobenzoyl-amino-acids (Table 2) were prepared in yields of >80% by the following procedure. Solutions of *S*-thiobenzoylmercaptoacetic acid [13] (1·06 g., 0·005 mole) in ether (20 ml.) and of the amino-acid (0·005 mole) in N-sodium hydroxide solution (10 ml.) were mixed, and the mixture was shaken vigorously for a few seconds. Additional equivalents of sodium hydroxide solution were added where amino-acids were used as their salts, *e.g.*, hydrochlorides of histidine and of lysine, or where the amino-acid side-chain included an acidic function, *e.g.*, aspartic acid, glutamic acid, tyrosine. The mixture was then kept at room temperature for 2—12 hr., the change towards pale yellow in the colour of the aqueous phase indicating the reaction rate, and was then acidified to Congo Red with 2N-sulphuric acid. The mixture was extracted with ether (the insoluble derivatives of histidine and of lysine were precipitated on neutralisation), and the ethereal extracts were combined, dried ($MgSO_4$) after thorough washing with water, and evaporated *in vacuo*. The *N*-thiobenzoylamino-acid was conveniently isolated from the crude product by chromatography on silica gel. Development with benzene, followed by benzene–ether (20 : 1) caused successive elution of mercaptoacetic acid and of residual *S*-thiobenzoyl-mercaptoacetic acid; the pale yellow *N*-thiobenzoylamino-acid was then eluted with ether.

Cyclohexylammonium salts of the derivatives were prepared by the procedure of Sjöberg, Karlen, and Dahlbom.[3] ($\varepsilon_l - \varepsilon_r$) values quoted in Table 2 were obtained with crystalline cyclohexylammonium salts, with crystalline *N*-thiobenzoylamino-acids, and with acids liberated from their crystalline salts by extraction with ether from acidified aqueous solutions.

N-*Thiobenzoyl*-DL-*alanine.*—A solution of *S*-thiobenzoylmercaptoacetic acid (2·12 g.) and L-alanine (0·89 g.) in N-sodium hydroxide (20 ml.) was warmed at 50—60° during 2 hr. The resulting pale yellow solution was cooled, made acid to Congo Red, and extracted with ether. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated. The residue (1·90 g.) yielded *N*-thiobenzoyl-DL-alanine (0·712 g.), m. p. 124° (lit.,[10] 124—125°) (from benzene–light petroleum). It follows that racemisation had occurred to the extent of at least 40%. The mother-liquors were evaporated; the residual oil, in ether, showed considerable circular dichroism at 395 mμ.

Thiobenzamide.—Prepared by the procedure of Fairfull, Lowe, and Peak,[14] m. p. 118·5°

[10] B. Holmberg, in "The Svedberg, 1884—1944," Almqvist and Wiksells, Uppsala, 1944, p. 299.
[11] A. Kjaer, *Acta Chem. Scand.*, 1950, **4**, 1347.
[12] Cf. J. Goerdeler and H. Horstmann, *Chem. Ber.*, 1960, **93**, 671.
[13] F. Kurzer and A. Lawson, *Org. Synth.*, 1962, **42**, 100.
[14] A. E. S. Fairfull, J. L. Lowe, and D. A. Peak, *J.*, 1952, 742.

TABLE 2

N-Thiobenzoylamino-acids

No.	Amino-acid	M. p.	Cryst. from	Anisotropic $\lambda_{max.}$ (mμ) [a] Ether	Anisotropic $\lambda_{max.}$ (mμ) [a] MeOH	Circular dichroism Ether $(\varepsilon_l - \varepsilon_r)$	Circular dichroism Ether $\lambda_{max.}$	Circular dichroism MeOH $(\varepsilon_l - \varepsilon_r)$	Circular dichroism MeOH $\lambda_{max.}$
1	L-Alanine	Oil	—	393	376	−1·32	391	−0·07	365
2	cyclohexyl-ammonium salt	135—136°	$CH_3{\cdot}CO_2Et$	—	370 [b]	—	—	−0·75	370
3	L-Aspartic acid	Oil	—	393	375	−0·59	392	+0·10	380
4	biscyclohexyl-ammonium salt	201—202	EtOH	—	370 [b]	—	—	+0·15	392
5	L-Glutamic acid	109—110 [c]	$CH_3{\cdot}CO_2Et$–C_6H_6	395	377	−0·56	387	+0·36	390
6	biscyclohexyl-ammonium salt	221—222 [d]	EtOH–Et_2O	—	367 [b]	—	—	+0·24	380
7	D-Glutamic acid	109—110	$CH_3{\cdot}CO_2Et$–C_6H_6	395	377	+0·54	389	−0·36	390
8	biscyclohexyl-ammonium salt	216—218	EtOH–Et_2O	—	370 [b]	—	—	−0·26	380
9	Glycine	152 [e]	MeOH–H_2O	394	376	—	—	—	—
10	cyclohexyl-ammonium salt	106	EtOH–Et_2O	—	369 [b]	—	—	—	—
11	L-Histidine	210—212	H_2O	—	377	—	—	−0·49	373
12	cyclohexyl-ammonium salt	130—131	$CHCl_3$–Et_2O	—	370 [b]	—	—	+0·10	385
13	Hydroxy-L-proline	168	$CH_3{\cdot}CO_2Et$	393	374	−2·42	395	−2·43	378
14	cyclohexyl-ammonium salt	220	EtOH–Et_2O	—	367	—	—	−1·54	375
15	L-Leucine	103—104 [f]	C_6H_6–pet. [g]	394	376	−0·83	389	+1·26	375
16	cyclohexyl-ammonium salt	164 [h]	EtOH–Et_2O	—	370 [b]	—	—	+0·49	372
17	L-Lysine	258 [i]	H_2O	—	—	—	—	—	—
18	L-Phenylalanine	Oil	—	396	376	−0·06	360—390	+1·43	377
19	cyclohexyl-ammonium salt	122	Et_2O	—	368 [b]	—	—	+0·38	375
20	L-Proline	Oil	—	391	368	−0·38	402	−0·29	390
21	cyclohexyl-ammonium salt	214—215 [j]	EtOH–Et_2O	—	357	—	—	−0·15	395
22	L-Serine	Oil	—	394	377	−1·22	396	−0·14	375
23	cyclohexyl-ammonium salt	170	EtOH–Et_2O	—	377	—	—	−1·05	372
24	D-Serine	Oil	—	394	377	+1·29	395	+0·16	375
25	cyclohexyl-ammonium salt	171	EtOH–Et_2O	—	376	—	—	+0·97	372
26	L-Tyrosine	Oil	—	395	375	−0·04	360—390	+1·52	375
27	cyclohexyl-ammonium salt	161	$CH_3{\cdot}CO_2Et$	—	370 [b]	—	—	+0·37	376
28	L-Valine	Oil	—	395	377	−0·61	394	+0·10	399
29	cyclohexyl-ammonium salt	176—177	$CH_3{\cdot}CO_2Et$	—	369 [b]	—	—	+0·10	398

No.	Found (%) C	H	N	S	Formula	Required (%) C	H	N	S
2	62·15	7·8	8·65	10·95	$C_{16}H_{24}N_2O_2S$	62·3	7·85	9·1	10·4
4	61·0	8·15	9·5	6·8	$C_{22}H_{37}N_3O_4S$	61·15	8·25	9·35	7·1
7	53·95	4·85	5·6	11·8	$C_{12}H_{13}NO_4S$	53·9	4·8	5·25	12·0
8	61·85	8·35	9·5	7·2	$C_{24}H_{39}N_3O_4S$	61·9	8·45	9·05	6·9
10	60·55	7·25	9·55	11·15	$C_{15}H_{22}N_2O_2S$	61·2	7·55	9·5	10·9
11	56·8	4·8	15·45	11·35	$C_{13}H_{13}N_3O_2S$	56·7	4·75	15·25	11·65
12	60·3	6·95	14·45	8·6	$C_{19}H_{26}N_4O_2S$	60·95	7·0	14·95	8·55
13	57·3	5·2	5·7	12·6	$C_{12}H_{13}NO_3S$	57·35	5·2	5·55	12·75
14	61·65	7·45	8·1	9·05	$C_{18}H_{26}N_2O_3S$	61·7	7·5	8·0	9·15
15	62·05	6·7	5·6	12·55	$C_{13}H_{17}NO_2S$	62·1	6·8	5·55	12·75
17	58·7	6·95	10·7	11·75	$C_{13}H_{18}N_2O_2S$	58·6	6·8	10·5	12·05
19	68·4	7·15	6·95	7·8	$C_{22}H_{28}N_2O_3S$	68·7	7·35	7·3	8·35
23	59·0	7·45	8·75	9·9	$C_{16}H_{24}N_2O_3S$	59·25	7·45	8·65	9·9
25	59·45	7·4	8·65	9·9	$C_{16}H_{24}N_2O_3S$	59·25	7·45	8·65	9·9
27	65·4	6·85	7·3	8·1	$C_{22}H_{28}N_2O_3S$	65·95	7·05	7·0	8·0
29	64·1	8·6	8·4	9·5	$C_{18}H_{28}N_2O_2S$	64·25	8·4	8·35	9·55

[a] log ε ~2·3. [b] Inflection. [c] Lit.,[10] 111—112°. [d] Lit.,[3] 207—210°. [e] Lit.,[10] 150—151°. [f] Lit.,[3] oil. [g] pet = light petroleum (b. p. 40—60°). [h] Lit.,[3] 159—161°. [i] Decomp. [j] Lit.,[3] 215—216°.

(lit.,[14] 116°), this had $\lambda_{max.}$ (ether) 404 mμ (log ε 2·38), $\lambda_{infl.}$ (MeOH) 386 mμ (log ε 2·38). The low-intensity absorption features in these spectra were unaffected when cyclohexylamine was added to the solutions.

Hippuric Acid from N-*Thiobenzoylglycine.*[12]—Aqueous 2N-silver nitrate (1 ml.) was added to a solution of *N*-thiobenzoylglycine (0·195 g., 0·001 mole) in acetone (20 ml.). The colourless precipitate, which was formed immediately, turned black on standing or more rapidly on gentle warming of the mixture, and was then filtered off. Water (1 ml.) was added to the colourless filtrate, which was then concentrated to 1 ml.; hippuric acid (0·151 g.), m. p. 184—185° (m. p. undepressed on admixture with authentic material, m. p. 187°), crystallised from the concentrated filtrate after it had been diluted with ethanol (2 ml.).

N-Thiobenzoyl-DL-alanine was similarly converted into *N*-benzoyl-DL-alanine, m. p. 163—164° (lit.,[15] 165—166°).

The author thanks Professor H. N. Rydon for his interest; and Professor S. F. Mason and Dr. A. J. McCaffery for helpful discussions.

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[15] J. Baum, *Z. physiol. Chem.*, 1885, **9**, 465.