CCA - 149

547.279.52(547.466.3-261):541.632

The Resolution of β-Amino-γ-methylsulphinyl-butyric Acid (β-Methionine Sulphoxide) into Four Optical Isomers

D. Keglević and B. Ladešić

Tracer Laboratory, Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

Received May 9, 1959

The preparation of four optical isomers of β-amino-γ-methylsulphinyl-butyric acid (β-methionine sulphoxide) is described. *S*-Benzyl-DL-β-homocysteine was resolved *via* its brucine salt into the optically active forms (IIa and IIb) which were then converted to L- resp. D-β-methionine (IIIa and IIIb). By desulphurization with Raney-Nickel to the corresponding β-amino acids (IVa and IVb), the optical purity of IIIa and IIIb was confirmed. Oxidation of IIIa and IIIb with hydrogen peroxide yielded the diastereoisomeric mixtures of L- resp. D-β-methionine sulphoxides; they were resolved into four optical isomers through picrates resp. through fractional crystallization. The rotation values of pure isomers as well as percentages of isomers occurring in the diastereoisomeric mixtures are given.

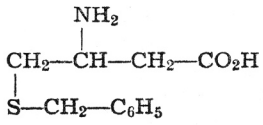
β-Amino-γ-methylsulphinyl-butyric acid (β-methionine sulphoxide, V) was established as a urinary metabolite of β-amino-γ-methylthio-butyric acid (β-methionine) in rats fed with methyl-^{14}C-labelled β-methionine[1]. It is known that sulphoxides, bearing two different groups at the sulphur atom, can exhibit optical activity, the unshared pair of sulphur electrons acting as the fourth group in the corner of the tetrahedron. Only a few compounds of this type, being of biological and biochemical interest, have been described so far. Alliin[2] isolated from garlic, as well as sulphoraphene[3] and 4-methyl-sulphoxydebuten-3-yl-cyanide[3] isolated from radish, proved to be optically active sulphoxides, the latter two owing its activity only to the asymmetry of the sulphur atom. Alliin and sulphoraphene were also synthetically prepared and resolved into optically active forms. Lavine[4] succeeded in resolving diastereoisomers of L-methionine sulphoxide through the picrates; it was shown[5] that they had significantly different antimetabolic activities, indicating thus enzymic sensitivity to the asymmetry of the sulphur atom. Biotin sulphoxide was also resolved into two diastereoisomeric forms[6], one of which proved to be identical with crystalline AN factor, isolated from *Aspergillus niger* culture filtrate[7].

Synge and Wood[8] isolated *S*-methyl-L-cysteine-(+)-sulphoxide from cabbage. Recently[9] the isolation of a new sulphoxy amino acid cycloalliin from onion has been reported.

In connection with further work on the metabolism of labelled β-methionine, it seemed to us of interest to prepare all four optical isomers of β-methionine sulphoxide, starting from L- resp. D-β-methionine.

L-β-methionine was synthesized by Balenović[10] using *S*-benzyl-L-β-homocysteine[11,12] as the starting material. The configuration of these β-amino acids was also established[13]. DL-β-methionine was obtained by Birkofer[14] from methyl γ-benzylthio-crotonate *via* *S*-benzyl-DL-β-homocysteine[15].

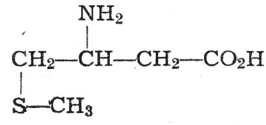
The best way leading to the preparation of both isomers of β-methionine seemed to be in the optical resolution of *S*-benzyl-DL-β-homocysteine. Therefore, the *N*-formyl derivative of *S*-benzyl-DL-β-homocysteine (I) was synthesized and the fractional crystallization of its strychnine and brucine salts in different solvents was tried. The brucine salt obtained from 40% acetone proved to give the best resolution, yielding *S*-benzyl-L- resp. D-β-homocysteine (IIa and IIb), with $[\alpha]_D - 64^0$ and $[\alpha]_D + 60^0$.



II

a, C_β = L-

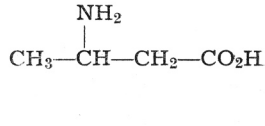
b, C_β = D-



III

a, C_β = L-

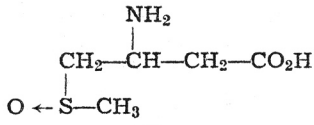
b, C_β = D-



IV

a, C_β = L-

b, C_β = D-



V

a, L-(+)
b, L-(—)
c, D-(—)
d, D-(+)

IIa and IIb were converted by the already published method[10,1] into L- and D-β-methionine (IIIa and IIIb), showing $[\alpha]_D - 24^0$ and $[\alpha]_D + 24^0$ respectively. IIIa proved to be optically identical with L-β-methionine prepared from L-cystine as the starting material[10].

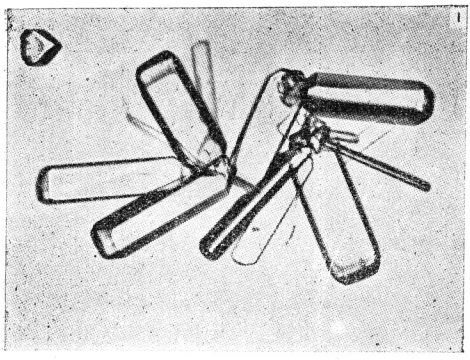
In order to check the optical purity of L- and D- β-methionine, both compounds were converted by desulphurization with Raney-Nickel into the corresponding β-aminobutyric acids (IVa and IVb). The rotation values obtained, agreed with those reported by Fischer[16] for (+) and (—) β-aminobutyric acid, thus indicating that the resolution of *S*-benzyl-DL-β-homocysteine was complete.

Oxidation of L- and D- β-methionine with hydrogen peroxide in glacial acetic acid, gave high yields of L- and D-β-methionine sulphoxides (Va, b and Vc, d). The resolution of diastereoisomers was tried in two ways: *A.* through the picrates, following in general the procedure given by Lavine[4], and *B.* by fractional crystallization. The latter gave better yields as well as slightly higher rotation values. Va, resp. Vc crystallized from 80% ethanol and showed to be less soluble in ethanol than Vb, resp. Vd, which precipitated from ethanolic solutions after addition of absolute ethanol and acetone. The properties of the four isomers are summarized in Table I; Va and Vc on the one hand, and Vb and Vd on the other, are optical antipodes.

TABLE I

Properties of four optical isomers of β-Methionine sulphoxide

Nr.	Substance	m. p.	Specific rotation (c, 0.8, 1 dm.) $[\alpha]_D$		Crystals from
			50% methanol	*N* HCl	
Va	L-β-Methionine-(+)-sulphoxide	200—201°	+153.1°	+139.0°	Prisms 80% ethanol
Vb	L-β-Methionine-(—)sulphoxide	184—185°	— 57.7°	— 70.7°	Needles ethanol/acetone
Vc	D-β-Methionine-(—)sulphoxide	199—200°	—153.7°	—139.6°	Prisms 80% ethanol
Vd	D-β-Methionine-(+)-sulphoxide	184—185°	+ 58.5°	+ 72.3°	Needles ethanol/acetone



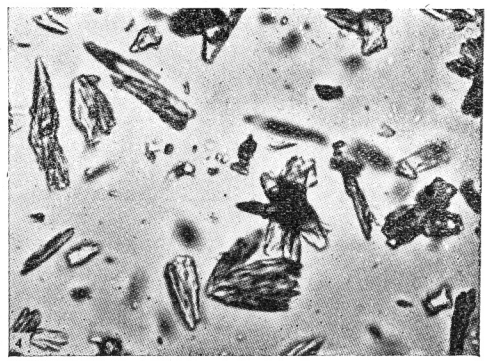


Fig. 1—2. Microphotographs of
1. L-β-Methionine-(+)-sulphoxide; magn. 150×
2. D-β-Methionine-(+)-sulphoxide; magn. 240×

The infrared spectra of the four isomers were also examined. Two of them are presented in Fig. 3. The enantiomorphic pair Va and Vc showed to have identical spectra. The second enantiomorphic pair Vb and Vd exhibited a difference in the intensity of characteristic »amino acid bands I and II« appearing at 1530 and 1610 cm^{-1}, those of Vd (D-(+)-sulphoxide) being very weak. All four isomers exhibited absorption band at 1015—995 cm^{-1} which is attributed to S=O link, and a second band appearing at 705—695 cm^{-1} which is ascribed to the C—S—C linkage. There was no significant difference between diastereoisomers regarding the position of the absorption bands characteristic of functional groups, but some differences in the frequencies appearing in the 1300—1000 cm^{-1} region were observed.

Oxidation by hydrogen peroxide did not result in equimolar amounts of the two diastereoisomers; this could be seen from the yields of pure isomers obtained by fractional crystallization, as well as by comparing the rotation

values of the diastereoisomeric mixture with the rotation values of pure isomers. Lavine[4] has already shown in the case of L-methionine that different methods of oxidation, which produce the second centre of asymmetry, perform varying degrees of asymmetric synthesis. Presumably this is a result of varying rates of reaction. Lavine[4] tried the oxidation by several agents, but in the case of hydrogen peroxide he obtained almost equimolar amounts of (+) and (—) L-methionine sulphoxides.

As observed in six independent hydrogen peroxide oxidations, carried under the same conditions, with L- resp. D- β-methionine, the rotation values of diastereoisomeric mixtures varied only slightly, indicating that no significant differences in the ratio of particular isomers occurred.

Table II shows the calculated percentages of isomers in the obtained diastereoisomeric mixtures Va, b and Vc, d. The values indicate clearly that

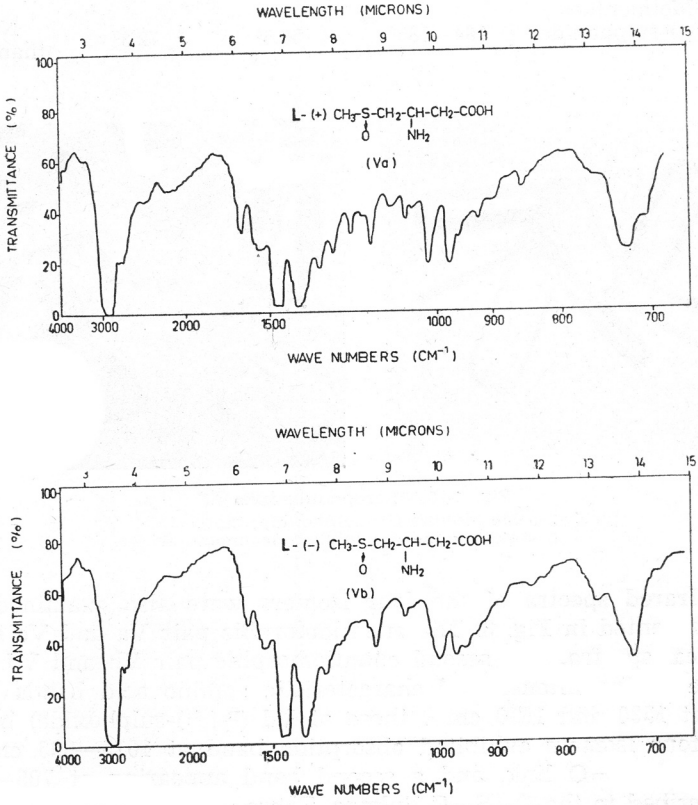


Fig. 3. Infrared absorption spectra in Nujol (Perkin-Elmer Mod. 134 spectrophotometer NaCl prisms)

the oxidation proceeded in favour of Vb resp. Vd, which are optical antipodes. Thus, under the conditions described, the enantiomorphic pair Vb and Vd compared with the enantiomorphic pair Va and Vc was produced in a ratio of roughly 3:1.

TABLE II

Molar rotations and percentages of isomers in the diastereoisomeric mixtures of β-Methionine sulphoxide

Substance	$[M]_D$ of diastereoisomeric mixture actually obtained by the oxidation		$[M]_D$ of pure isomer		Calculated % of isomers in diastereoisomeric mixture		$[M]_D$ of diastereoisomeric mixture prepared from optically pure isomers	
	50% methanol	*N* HCl	50% methanol	*N* HCl	50% methanol	*N* HCl	50% methanol	*N* HCl
L-β-Methionine--(+)-sulphoxide	$0^0 \pm 3$	$-21.5^0 \pm 2$	$+252.9^0 \pm 2$	$+229.6^0 \pm 2$	27.4	27.5	$0^0 \pm 3$	$-23.1^0 \pm 3$
L-β-Methionine--(—)-sulphoxide			$-95.3^0 \pm 2$	$-116.8^0 \pm 2$	72.6	72.5		
D-β-Methionine--(—)-sulphoxide	$0^0 \pm 3$	$+16.5^0 \pm 2$	$-253.9^0 \pm 2$	$-230.6^0 \pm 2$	27.6	29.4	$0^0 \pm 3$	$+19.2^0 \pm 3$
D-β-Methionine--(+)-sulphoxide			$+96.6^0 \pm 2$	$+119.4^0 \pm 2$	72.4	70.6		

From the optically pure isomers, taken at the same ratio as the calculations showed to occur by oxidation, diastereoisomeric mixtures were prepared. Within the limits of experimental error, they showed to have the same molar rotations as the diastereoisomeric mixtures obtained in the experiment.

EXPERIMENTAL

All melting points are uncorrected

S-Benzyl-DL-β-N-formyl-homocysteine (I)

Acetic formic anhydride was prepared according to Huffman[17] from acetic acid anhydride (12.2 ml.) and formic acid (98%, 5.2 ml.). *S*-Benzyl-DL-β-homocysteine[15] (13.5 g., 60 mMoles) was gradually added for half an hour to the stirred mixture of anhydride, maintained at 50—60°, and the solution was kept under the same conditions for additional five hours. Water (10 ml.) was added and the solution evaporated to dryness *in vacuo*. The residue was dissolved in hot ethylacetate, treated with charcoal, filtered and cooled to 0°. 12.3 g. (81%) of I separated as white crystals m. p. 70—72°. Additional 1.5 g. was obtained from mother liquor on addition of petroleum-ether (total yield 91%).

The analytical sample, recrystallized twice from ethylacetate, showed m. p. 83—84.5°.

Anal. 6.92 mg. subst.: 14.40 mg. CO_2, 3.65 mg. H_2O
5.69 mg. subst: 0.275 ml. N_2 (20°, 745 mm)
$C_{12}H_{15}NO_3S$ (253.32) calc'd.: C 56.89; H 5.97; N 5.53%
found : C 56.83; H 6.03; N 5.52%

The resolution of S-benzyl-DL-β-N-formyl-homocysteine with brucine

12.6 g. (50 mMoles) of I and 21.0 g. (53.5 mMoles) of anhydrous brucine were dissolved in hot 40% acetone (100 ml.) and the solution cooled in the refrigerator for 24 hours. 16.9 g. of brucine salt showing $[\alpha]_D^{24} -18.2° \pm 1$ (c, 1.92 in ethanol) was collected. The salt was recrystallized from water (600 ml.) yielding 11.9 g. of white crystals with $[\alpha]_D^{24} -16.5° \pm 1$ (c, 1.97 in ethanol). The second crystallization from water (150 ml.) resulted in a product (11.1 g., 65.5%) m. p. 65—70° with no change in rotation: $[\alpha]_D^{25} -16.3° \pm 1$ (c, 2.00 in ethanol). From water mother liquors, after evaporation to the half of volume, an additional crop of 1.5 g. (total yield: 74.5%) having the same rotation was obtained. Analysis showed that the salt crystallized with one and half molecule of water.

Anal. 5.73 mg. subst.: 13.10 mg. CO_2, 3.41 mg. H_2O
4.31 mg. subst.: 0.233 ml. N_2 (20°, 748.5 mm)
$C_{35}H_{41}N_3O_7S \cdot 1.5\ H_2O$ (674.79)
calc'd.: C 62.29; H 6.57; N 6.23%
found : C 62.42; H 6.67; N 6.21%

S-Benzyl-L-β-homocysteine (IIa)

The brucine salt (11.1 g., $[\alpha]_D^{24} -16.3°$, in ethanol) was shaken with chloroform (25 ml.) and 1 *N* ammonium hydroxide (50 ml.) in a separatory funnel. The aqueous layer was extracted with three portions of chloroform (10 ml.) and then concentrated *in vacuo* (to about 10 ml.). Concentrated hydrochloric acid was added to make the solution approximately 1*N*. To hydrolyze the formyl derivative, the solution was refluxed for one hour, cooled, and evaporated to dryness. The residue was dissolved in hot water (20 ml.) and neutralized with conc. ammonia (*p*H 6—7). After standing overnight in the refrigerator 3.15 g. (85% calc'd. on brucine salt, 55.9% calc'd. on I) of IIa were collected showing $[\alpha]_D^{25} -59.0° \pm 1$ (c, 1.12 in *N* HCl). One recrystallization from ethanol: water (4:1) raised the rotation to $[\alpha]_D^{25} -64.0 \pm 1$ (c, 1.16 in *N* HCl), m. p. 171—174° (decomp.).

S-*Benzyl*-D-β-*homocysteine* (IIb)

The acetone mother liquor from the brucine salt of I was evaporated to dryness *in vacuo*. The remaining oil (20.8 g.) was decomposed as described for the L-isomer. 5.65 g. of crude product was obtained showing $[\alpha]_D^{24} + 24.8^0 \pm 2$ (c, 1.11 in *N* HCl). After two recrystallizations from absolute ethanol 2.05 g. (36.5% calc'd. on I) of IIb with $[\alpha]_D^{24} + 60.5^0 \pm 1$ (c, 1.17 in *N* HCl) was obtained, m. p. 173—175° (decomp.).

Anal. 6.14 mg. subst.: 13.15 mg. CO_2, 3.78 mg. H_2O
$C_{11}H_{15}NO_2S$ (225.30) calc'd.: C 58.64; H 6.71%
found : C 58.42; H 6.88%

L-β-*Methionine* (IIIa)

Prepared by the already described procedure[10,1]. After two recrystallizations from absolute ethanol it had m. p. 166—167° (decomp.). The optical activity of the product, $[\alpha]_D - 24.1^0 \pm 1$ (c, 1.37 in water) was identical with the rotation of L-β-methionine (m. p. 166—167°, $[\alpha]_D^{20} - 23^0 \pm 1$) prepared by Balenović[10] from L-cystine.

D-β-*Methionine* (IIIb)

Prepared as described for L-isomer[10,1]. From 826 mg. (3.7 mMoles) of IIb ($[\alpha]_D^{24} + 60.5^0$, in *N* HCl) 366 mg. (66%) of IIIb was obtained as colourless prisms, showing $[\alpha]_D^{24} + 20.9^0 \pm 1.5$ (c, 1.29 in water). After one recrystallization from absolute ethanol, the substance showed $[\alpha]_D^{24} + 24.8^0 \pm 1$ (c, 1.29 in water) m. p. 166—168° (decomp.).

Anal. 5.30 mg. subst.: 7.83 mg. CO_2, 3.57 mg. H_2O
$C_5H_{11}NO_2S$ (149.21) calc'd.: C 40.25; H 7.43%
found : C 40.34; H 7.54%

L-β-*Aminobutyric acid* (IVa)

In general the procedure for desulphurization of α-methionine was followed[18]. A solution of IIIa (50 mg., 0.3 mMoles, $[\alpha]_D^{24} - 23.3^0$, in water) in 50% ethanol (4 ml.) was heated with about 1 g. of Raney-Nickel catalyst W-2[19] in ethanol at 90—95° for one hour. The precipitate was removed by filtration, washed with ethanol and the combined filtrates evaporated to dryness *in vacuo*. The oily residue was recrystallized from methanol-absolute ether. White prisms separated, m. p. 210—212°, $[\alpha]_D^{23} + 37.2^0 \pm 2$ (c, 0.3 in water). Fischer[16] reported for (+)-β-aminobutyric acid: m. p. 220°, $[\alpha]_D + 35.3^0$ (c, 9.6% in water).

D-β-*Aminobutyric acid* (IVb)

Prepared in the same way as the L-isomer. 91 mg. (0.6 mMoles) of IIIb yielded, after recrystallization from methanol-abs. ether, 11 mg. of analytically pure IVb, m. p. 210—212°, $[\alpha]_D^{23} - 38.5^0 \pm 1$ (c, 0.83 in water). Fischer[16] reported for (—)-β-aminobutyric acid, $[\alpha]_D - 35.2^0$ (c, 10% in water).

Anal. 3.43 mg. subst.: 6.02 mg. CO_2, 2.75 mg. H_2O
$C_4H_9NO_2$ (103.12) calc'd.: C 46.59; H 8.80%
found : C 46.60; H 8.74%

L-β-*Methionine sulphoxide* (L-β-*amino*-γ-*methylsulphinylbutyric acid*) (Va, b)

To a solution of L-β-methionine (IIIa, 225 mg., 1.5 mMoles, $[\alpha]_D^{23} - 23.2^0$, in water) in glacial acetic acid (2 ml.), 30% hydrogen peroxide (0.6 ml., 6 mMoles) was added with shaking. The mixture was allowed to stand at room temperature for four hours and then evaporated to dryness *in vacuo*. The remaining viscous oil was triturated with acetone (5 ml.) and kept at 0° overnight. White crystals 236 mg.

(95%) of Va, b separated, m. p. 172—173° (decomp.), $[\alpha]_D^{23} - 13.0° \pm 1$ (c, 0.82 in *N* HCl), $[\alpha]_D^{24}$ 0° ± 2 (c, 0.82 in 50% methanol).

In three independent experiments, carried under the same conditions, the optical rotation measured in *N* HCl gave values ranging from $[\alpha]_D - 10°$ up to $[\alpha]_D - 13°$.

D-β-*Methionine sulphoxide* (D-β-*amino*-γ-*methylsulphinylbutyric acid*) (Vc, d)

Prepared in the same way as L-isomer, from 375 mg. (2.5 mMoles) of IIIb ($[\alpha]_D^{23} + 23.1°$, in water). Yield: 386 mg., 93% of Vc, d showing m. p. 172—173° (decomp.) and $[\alpha]_D^{23} + 10.0° \pm 1$ (c, 0.83 in *N* HCl), $[\alpha]_D^{22}$ 0° ± 2 (c, 0.83 in 50% methanol).

In three independent experiments, carried under the same conditions, the optical rotation measured in *N* HCl gave values ranging from $[\alpha]_D + 10°$ up to $[\alpha]_D + 12.5°$.

A. Resolution of L⁻ *and* D⁻ β-*Methionine sulphoxides* via *picrates*

L-β-*Methionine*-(+)-*sulphoxide* (Va). 221 mg. (1.34 mMoles) of L-β-methionine-(±)-sulphoxide (Va, b) and 313 mg. (1.37 mMoles) of picric acid were dissolved in hot ethanol (10 ml.). On cooling at 0°, yellow crystals separated (350 mg., m. p. 159—161°). Recrystallization from ethanol yielded 231 mg. of Va picrate with m. p. 169—171°. Repeated crystallization did not change the m. p.

Anal. 5.97 mg. subst.: 7.35 mg. CO_2, 1.97 mg. H_2O
$C_{11}H_{14}N_4O_{10}S$ (394.31) calc'd.: C 33.50; H 3.58%
found : C 33.60; H 3.71%

Decomposition of Va picrate with amylamine after Lavine[4] yielded L-(+)-sulphoxide Va, which after two recrystallizations from 85% acetone showed $[\alpha]_D^{24} + 136.4° \pm 2$ (c, 0.88 in 50% methanol).

L-β-*Methionine*-(—)-*sulphoxide* (Vb). The ethanolic mother liquor containing the soluble picrate of Vb was evaporated to dryness *in vacuo*, and decomposed with amylamine as described above. Vb was obtained showing $[\alpha]_D^{24} - 47.0° \pm 2$ (c, 0.38 in 50% methanol).

D-β-*Methionine*-(—)-*sulphoxide* (Vc). Following the procedure for L-isomer from 206 mg. (1.25 mMoles) of D-β-methionine-(±)-sulphoxide (Vc, d), 197 mg. of Vc picrate was obtained. After two recrystallizations from ethanol the substance had m. p. 170—171°.

Anal. 1.60 mg. subst.: 1.97 mg. CO_2, 0.52 mg. H_2O
$C_{11}H_{14}N_4O_{10}S$ (394.31) calc'd.: C 33.50; H 3.58%
found : C 33.60; H 3.64%

Decomposition of picrate of Vc with amylamine yielded D-(—)-sulphoxide Vc. After recrystallization from 96% ethanol it showed $[\alpha]_D^{23} - 137.5° \pm 1$ (c, 0.96 in 50% methanol).

D-β-*Methionine*-(+)-*sulphoxide* (Vd). The ethanolic mother liquor containing the more soluble picrate of Vd was evaporated to dryness *in vacuo* and following the procedure for L-isomer, D-(+)-sulphoxide Vd, showing $[\alpha]_D^{23} + 55.4° \pm 2$ (c, 0.9 in 50% methanol), was obtained.

B. Resolution of L- *and* D- β-*Methionine sulphoxides by fractional crystallization*

L-β-*Methionine*-(+)-*sulphoxide* (Va). 294 mg. of L-β-methionine-(±)-sulphoxide (Va, b, $[\alpha]_D^{23} - 13°$, in *N* HCl) was dissolved in hot 80% ethanol (8 ml.) and allowed to stand in the refrigerator overnight. 88 mg. (29.9%) of Va crystallized, showing $[\alpha]_D^{23} + 110.8° \pm 2$ (c, 0.82 in 50% methanol). The recrystallization from 80% ethanol yielded 55 mg. of white crystals with $[\alpha]_D^{23} + 152.5° \pm 1$ (c, 0.82 in 50% methanol).

Analytical sample was once more recrystallized from 80% ethanol and showed m. p. 200—201° (decomp.) $[\alpha]_D^{23}$ + 153.1° ± 1 (c, 0.82 in 50% methanol), $[\alpha]_D^{23}$ + 139.0° ± 1 (c, 0.83 in *N* HCl).

Anal. 5.63 mg. subst.: 7.49 mg. CO_2, 3.38 mg. H_2O
$C_5H_{11}NO_3S$ (165.21) calc'd.: C 36.35; H 6.71%
found : C 36.30; H 6.71%

L-β-*Methionine-(—)-sulphoxide* (Vb). To the mother liquor of the first fraction, absolute ethanol was added till slight turbidity persisted. After standing overnight in the ice-box, 110 mg. of Vb having $[\alpha]_D^{24}$ — 43.1° ±2 (c, 0.79 in 50% methanol) was obtained. Aceton was added to the mother liquor and additional 57 mg. $[\alpha]_D^{23}$ — 48.8° ± 2 (c, 0.86 in 50% methanol) of Vb crystallized out. Total yield 167 mg. (56.8%). The combined crops were recrystallized from 90% acetone, and 143 mg., showing $[\alpha]_D^{23}$ — 49.7° ± 1 (c, 0.91 in 50% methanol), was obtained. A further recrystallization from the same solvent raised the rotation to $[\alpha]_D^{23}$ — 57.7° ±1 (c, 0.81 in 50% methanol), $[\alpha]_D^{23}$ — 70.7° ± 1 (c, 0.82 in *N* HCl) m. p. 184—185° (decomp.).

Anal. 6.30 mg. subst.: 8.38 mg. CO_2, 3.83 mg. H_2O
$C_5H_{11}NO_3S$ (165.21) calc'd.: C 36.35; H 6.71%
found : C 36.28; H 6.80%

D-β-*Methionine-(—)-sulphoxide* (Vc). 375 mg. of D-β-methionine-(±)-sulphoxide (Vc, d, $[\alpha]_D^{23}$ + 10°, in *N* HCl) was dissolved in hot 80% ethanol (8 ml.) and allowed to stand in the refrigerator overnight. 88 mg. (23.5%) of Vc having $[\alpha]_D^{23}$ — 132.5° ± 2 (c, 0.83 in 50% methanol) was obtained. After recrystallization from 80% ethanol, 54 mg. was collected $[\alpha]_D^{23}$ — 151.2° ± 1 (c, 0.82 in 50% methanol). A further recrystallization gave the product with $[\alpha]_D^{23}$ — 153.7° ± 1 (c, 0.82 in 50% methanol), $[\alpha]_D^{23}$ — 139.6° ± 1 (c, 0.83 in *N* HCl) m. p. 199—200° (decomp.).

Anal. 4.15 mg. subst.: 5.53 mg. CO_2, 2.54 mg. H_2O
$C_5H_{11}NO_3S$ (165.21) calc'd.: C 36.35; H 6.71%
found : C 36.35; H 6.84%

D-β-*Methionine-(+)-sulphoxide* (Vd). To the mother liquor of the first fraction absolute ethanol was added till turbidity persisted. After standing overnight in the ice-box, 193 mg. (51.5%) of Vd, showing $[\alpha]_D^{23}$ + 24.2° ± 1 (c, 0.83 in 50% methanol), was obtained. Acetone was added to the mother liquor and additional 61 mg. with $[\alpha]_D^{23}$ + 58.5° ± 1 (c, 0.82 in 50% methanol), $[\alpha]_D^{23}$ + 72.3° ± 1 (c, 0.82 in *N* HCl) m. p. 184—185° (decomp.) separated. The first precipitate (193 mg. $[\alpha]_D^{23}$ - 24.2) was recrystallized from 90% ethanol; 99 mg. of diastereoisomeric mixture having $[\alpha]_D^{23}$ + 4°±2 (c, 0.81 in 50% methanol) crystallized out, and when acetone was added to the filtrate till turbidity, 53 mg. with $[\alpha]_D^{23}$ + 53.1° ± 1 (c, 0.79 in 50% methanol) separated. Yield of optically pure Vd: 114 mg. (30.4%).

Anal. 5.17 mg. subst.: 6.90 mg. CO_2, 3.15 mg. H_2O
$C_5H_{11}NO_3S$ (165.21) calc'd.: C 36.35; H 6.71%
found : 36.41; H 6.81%

Preparation of diastereoisomeric mixtures

The calculated amounts of pure isomers were dissolved in appropriate solvent and measured in 0.5 dm. tubes.

L-β-*Methionine-(±)-sulphoxide* (Va, b)

A. In 50% *methanol:* Va (2.247 mg.) and Vb (5.876 mg.) showed $[\alpha]_D^{23}$ 0° ± 2 (c, 0.81).

B. In N *HCl:* Va (2.235 mg.) and Vb (5.887 mg.) showed $[\alpha]_D^{23}$ — 14.0° ± 2 (c, 0.81).

D-β-*Methionine-(±)-sulphoxide* (Vc, d)

A. In 50% *methanol:* Vc (2.206 mg.) and Vd (5.781 mg.) showed $[\alpha]_D^{23}$ 0° ± 2 (c, 0.80).

B. In N *HCl:* Vc (2.233 mg.) and Vd (5.362 mg.) showed $[\alpha]_D^{23}$ + 11.6° ± 2 (c, 0.76).

Acknowledgment. We are indebted to Dr. Z. Devidé (Laboratory for Electron Microscopy) for the preparation of photomicrographs and to Miss V. Oreški and Mr. F. Rudolf (Microchemical Laboratory — supervision Prof. L. Filipović) for the microanalyses. Thanks are also due to Dr. S. Ašperger for the help in the interpretation of the infrared spectra and to Mr. T. Magjer for their measurement.

REFERENCES

1. D. Keglević-Brovet, A. Kornhauser, B. Ladešić, S. Iskrić, N. Allegretti and L. Rabađija, *Croat. Crem. Acta* **29** (1957) 15.
2. A. Stoll und E. Seebeck, *Helv. Chim. Acta* **32** (1949) 197.
3. H. Schmid und P. Karrer, *Helv. Chim. Acta* **31** (1948) 1497.
4. T. F. Lavine, *J. Biol. Chem.* **169** (1947) 477.
5. E. Borek and H. Waelsch, *Arch. Biochem.* **14** (1947) 143.
6. D. B. Melville, *J. Biol. Chem.* **208** (1954) 495.
7. L. D. Wright, E. L. Cresson, J. Valiant, D. E. Wolf and K. Folkers, *J. Am. Chem. Soc.* **76** (1954) 4163.
8. R. L. M. Synge and J. C. Wood, *Biochem. J.* **64** (1956) 252.
9. A. J. Virtanen and E. J. Matikkala, *Acta Chem Scand.* **13** (1959) 623.
10. K. Balenović, D. Fleš and I. Jambrešić, *Croat. Chem. Acta* **28** (1956) 303.
11. K. Balenović and D. Fleš, *J. Org. Chem.* **17** (1953) 347.
12. K. Balenović, I. Jambrešić, B. Gašpert and Đ. Cerar, *Rec. trav. chim. Pays-Bas* **75** (1956) 1252.
13. K. Balenović, N. Bregant and Đ. Cerar, *J. Chem. Soc.* **1956**, 3982.
14. L. Birkofer und I. Hartwig, *Chem. Ber.* **87** (1954) 1189.
15. L. Birkofer und A. Birkofer, *Chem. Ber.* **89** (1956) 1226.
16. E. Fischer und H. Scheibler, *Liebigs Annal.* **383** (1911) 337.
17. C. W. Huffman, *J. Org. Chem.* **23** (1958) 727.
18. G. S. Fonken and R. Mozingo, *J. Am. Chem. Soc.* **69** (1947) 1212.
19. *Org. Syntheses, Coll, Vol.* **3** (1955) 181.

IZVOD

Rastavljanje β-amino-γ-metilsulfinil-maslačne kiseline (β-metionin sulfoksida) u četiri optička izomera

D. Keglević i B. Ladešić

Opisano je dobivanje četiriju optičkih izomera β-amino-γ-metilsulfinil-maslačne kiseline (β-metionin sulfoksida). *S*-Benzyl-DL-β-homocistein[15] rastavljen je preko brucinske soli u optički aktivne forme (IIa i IIb), koje su zatim prevedene u L-, odnosno D-β-metionin (IIIa i IIIb). IIIa i IIIb su desulfurirani Raney-Niklom u odgovarajuće β-aminomaslačne kiseline (IVa i IVb), pa je na taj način dokazana njihova optička čistoća. IIIa i IIIb oksidirani vodikovim peroksidom dali su diastereoizomerne smjese L-, odnosno D-β-metionin sulfoksida; preko pikrata, odnosno frakcioniranom kristalizacijom uspjelo je dobiti iz tih dviju diastereoizomernh smjesa četiri optička izomera. Navedena su skretanja tih izomera, a navedeni su i postoci, u kojima ih nalazimo u diastereoizomernoj smjesi.

TRACER LABORATORIJ
INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

Primljeno 9. svibnja 1959.