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QUANTITATIVE SEPARATION AND ANALYSIS OF DIASTEREOMERS OF L-METHIONINE-S,R-SULFOXIMINE VIA CYCLIC N-BLOCKED DERIVATIVES

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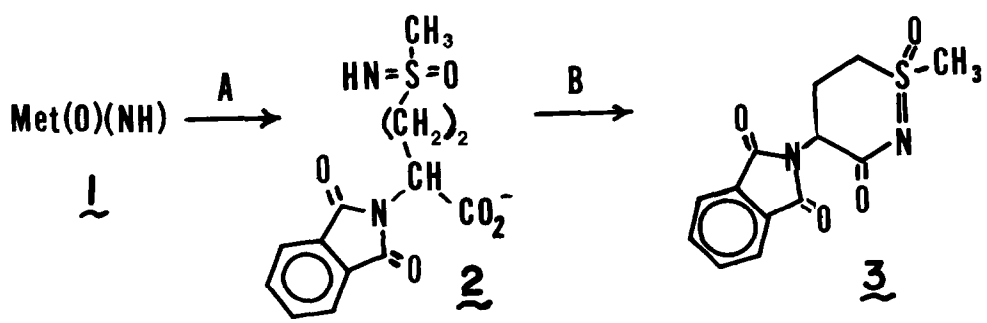
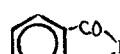
Summary: The diastereomers of L-methionine-S,R-sulfoximine, blocked at α-N with phthaloyl, then cyclized with Ac_2O to form 3,4,5,6-tetrahydro-1-methyl-3-oxo-4-(N-phthalimido)-1,2-thiazine-1-oxide, could be easily separated by fractional crystallization or HPLC, whereas the parent compounds were very difficult to separate.

Methionine sulfoximine (1) has been shown to be a potent active site inhibitor of glutamine synthetase *in vivo* and *in vitro* (1-5). It can act as both a transition state analog (6) and suicide inhibitor upon reaction with ATP. The L,S isomer is the only one of the four possible isomers that both binds tightly and irreversibly inhibits the enzyme (7). Proof of this rested upon unambiguous, complete separation of these isomers. The diasterieomers of commercially available L-methionime-S,R-sulfoximine are so polar and differ so little in physical properties as to be indistinguishable in their solubilities, chromatographic R_f values, or NMR chemical shifts and coupling constants. Only IR absorption bands in the "fingerprint" region served to distinguish these isomers (8,9) but could not be used to determine purity. Previous separations relied upon repetitive fractional recrystallizations with d-10-camphor-sulfonic acid (10), and provided at best only 95-98% separation. Synthesis of derivatives with decreased polarity was an obvious strategy, but in our hands the α-N-CBZ, α-N-phthaloyl, or α-benzyl ester derivatives offered no advantages and were difficult to isolate. Therefore, as a further step, the cyclized six-membered ring compounds were synthesized from the N-blocked derivatives:

SCHEME 1: Synthesis of 3



A = 

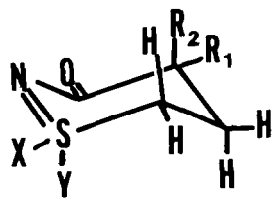
, 1.5 h/0°; 15 h/20°.

B = $(CH_3CO)_2O$, 6 h/20°, or $(C_6H_{11}\text{-}N=)_2C$, 3 h/0°; 9 h/20°; pH 3.

Analogous reactions for converting 2 to 3 have not been reported previously. Useful chemical results relevant to this work include preparation of a cyclic sulfoximine using a labile N-acetyl protecting group (11), the use of acetic anhydride to prepare 5- and 6- membered cyclic lactones from the relevant N-phthaloyl-L-amino acids (12,13), and conditions to promote or prevent racemization of N-phthaloyl-L-amino acids in pyridine (14). Unsuccessful procedures for preparation of 3 included: (a) reflux of 2 with polyphosphoric acid (15), that gave the cyclic lactone of N-phthaloyl-L-homoserine, due to cleavage of the S-C bond, and (b) reflux of 3a or 3b in DMF or pyridine, that gave racemization at the α-C, as indicated by multiple peaks on HPLC (Waters-C_{18}). Subsequent structural analysis of these fractions indicated that 3a → 3d and 3b → 3c.

The absolute configuration of 3a has been elucidated by Christensen et al. (10) by X-ray crystallography, and was correlated with IR data in the fingerprint region (8,9). Since the absolute stereochemistry of 3a is known, one can relate the physical parameters (Table I) and configurations of 3a - 3d (Scheme II) by well-defined chemical conversions; e.g., conversion of L-2 by Method B (Scheme I) gives L-3, but not D-3 or D,L-3. Isolation of 3a, followed by reflux in pyridine, gave 3a and 3d (D,L at α-C); similarly, racemization of 3b at α-C by pyridine reflux gave 3b and 3c. Hydrolysis and deblocking of isolated 3a gives only 1a, 3b gives only 1b, and so on.

SCHEME II: Stereochemistry of Isomers of 3



Cpd.	x	y	R_1	R_2
3 a (L,S)	Me	=O	N-Phth	H
3 b (L,R)	=O	Me	N-Phth	H
3 c (D,R)	=O	Me	H	N-Phth
3 d (D,S)	Me	=O	H	N-Phth

In practice, the HPLC method allowed us to separate with baseline resolution all the possible isomers of 3 (Scheme II) and provided a means of assessing purity and degrees of racemization (16).

Our rationale for the increased ease of separation of the isomers (e.g., 3a and 3b) runs as follows. X-ray analysis (10) showed that bond angles about the methyl sulfonimido group were close to tetrahedral (109°), except for the N-S-O angle of 118°. In the cyclic derivatives the larger groups are apt to be equatorial due to steric hinderance. The fact that the N-CBZ-cyclic derivatives were not so easily separable supports this view. This arrangement (Scheme II) also avoids an eclipsed conformation between α-C and β-C. Upon cyclization of 2 to 3 the asymmetric centers are 1,4 and effect each other relatively more stereochemically in 3 than in the acyclic forms, 1 or 2. Restriction of free rotation also may enhance these differences.

Table I shows various physical parameters observed for the separated isomers of 1 and 3. From the relationship between dihedral angles and observed coupling constants of 3 in ^{1}H-NMR,

conformationally the isomers resemble each other closely, except at the asymmetric S atom. The α-H, H_{5a} and H_{6a} protons adapt the axial conformation, whereas the H_{5e} and H_{6e} protons are equatorial. Variations of chemical shifts for H_{5a}, H_{6a}, and H_{6e} for 3a vs 3c and 3b vs 3d suggests either 1,3-diaxial interaction for H_{5a} and an oxygen atom or different configurations of the methyl sulfonimidoyl group (17,18).

TABLE I: Physical Properties of Isomers of 1 and 3

Cpd.	$[\alpha]_D^{24}$ [a]	CD ($\Delta\varepsilon_\lambda$)	MP (°C, dec. ± 2°)	
			obs.[e]	Lit(10)
1a (L,S)[b]	+ 34		239	239
1a[c]	+ 34		239	
1b (L,R)[b]	+ 39		235	235
1b[c]	+ 39		235	
1c (D,R)[c]	- 34		239	
(1b + 1d[c],)[d]	0		232	
3a		-	246	
3b		-	243	
3c		+	246	
3d		+	243	
(3b + 3d)[d]		0	316	

[a] (c = 2, 1 N HCl), ± 0.5°. Lit. $[\alpha]_D^{22}$ (c = 2, 1 N HCl): 1a, + 34°; 1b, + 39°. - ref. 10.

[b] via diastereomer of d-10-camphorsulfonic acid (Lit. 1a, 239°C; 1b, 235°C). - ref. 10.

[c] via cyclic compound.

[d] racemic compound.

[e] temperature increase rate: 40-70 sec/°C, using precision calibrated thermometers.

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9. IR (cm^{-1}) of L,S isomer: 1590, 1410, 1330, 1200, 1030, 870, and 820; of L,R isomer: 1640, 1545, 1410, 1350, 1215, 1020, and 840.

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16. HPLC separation experiments were carried out by Waters Associates, Milford, MA, with an analytical-scale instrument, using either a reverse-phase (C_{18}) or CN column with methanol/water solvents.

17. NMR (in DMSO) of 3a, 3b:

Chemical shifts(δ): C_6H_4 (7.96-780, 7.96-7.80), H_3 (4.92, 4.90), H_{6a} (3.83, 4.12), H_{6e} (4.22, 3.82), S-CH_3 (3.42, 3.42), H_{5a} (2.78, 3.00), H_{5e} (2.43, 2.50).

Coupling constants(Hz): $J_{H_4, H_{5a}}$ (11.6, 12.4), $J_{H_4, H_{5e}}$ (5.15, 5.0), $J_{H_{5a}, H_{6e}}$ (3.8, ca. 3), $J_{H_{5e}, H_{6a}}$ (3.6, 3.6), $J_{H_{5e}, H_{6e}}$ (4.14, ca. 4), $J_{H_{6a}, H_{6e}}$ (14.3, 13.1), $J_{H_{5a}, H_{5e}}$ and $J_{H_{5a}, H_{6a}}$ (>10).

18. CD data, Δελ (1 g/L, DMF, 24°C):

3a: -0.57, 311 nm; -0.40, 325 nm

3b: -0.51, 312 nm; -0.35, 322 nm; -0.36, 326 nm

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