cated the presence of a monofluoro derivative (5.68 μ). The material melted at 143–170°. It was sublimed to give 74.7 mg., m.p. 140–167°; $\lambda_{max}^{CHCl_3}$ 5.68, 9.96, 10.52, 10.63, 10.78, 10.95, 11.30, 11.54. The only absorption in the 9.9–11.6 μ region of comparable intensity in the parent ketone is at 9.94 μ, though approximately the same number of extremely weak bands, slightly shifted, appear.

3β-Acetoxy-16α-fluoroandrost-5-en-17-one (VIIb).—A solution of 5.0 mg. of 16α-fluoro-3β-hydroxyandrost-5-en-17-one in 2 drops of pyridine and 2 drops of acetic anhydride was allowed to stand at room temperature overnight, then diluted with water and the precipitate collected and recrystallized from methanol to give 1.7 mg. of VIIb, m.p. 199.5–201.5°. Material obtained by chromatography following acetylation of a crude product mixture from the fluorination of 3β-hydroxyandrost-5-en-17-one melted at 201–203°; mixture m.p. 200–202°. The infrared spectra (KBr pellet) were identical; $[\alpha]_D$ +18.8°.[22]

Anal. Calcd. for $C_{21}H_{29}FO_3$: C, 72.38; H, 8.39. Found: C, 72.72; H, 8.20.

(22) S. Nakanishi and E. V. Jensen [*J. Org. Chem.*, **27**, 702 (1962)] recently have described the preparation of this compound using perchloryl fluoride and a 16,17-en-17-amide. They report m.p. 205–206°, $[\alpha]_D$ ($CHCl_3$) + 14°. Their free alcohol had m.p. 182–183°, $[\alpha]_D$ ($CHCl_3$) + 18°.

The Synthesis of Some Acidic Amino Acids Possessing Neuropharmacological Activity

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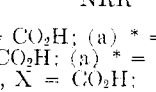
Several new acidic amino acids have been synthesized and tested for neuropharmacological action. *N*-Methyl- and *N*-ethyl-D-aspartic acid and D-homocysteic acid have potent excitatory actions upon mammalian and amphibian neurones.

The observation that L-aspartic and L-glutamic acids excite mammalian nerve cells and cause contraction of crustacean muscle[1–3] led to an extensive survey of the actions of related substances on mammalian and toad spinal neurones.[4–6] It was found that N-methyl-DL-aspartic acid was considerably more potent, both on

(1) J. Robbins, *J. Physiol. (London)*, **148**, 39 (1959).
(2) A. Van Harreveld, *J. Neurochem.*, **3**, 300 (1959).
(3) D. R. Curtis, J. W. Phillis and J. C. Watkins, *J. Physiol. (London)*, **150**, 656 (1960).
(4) D. R. Curtis and J. C. Watkins, *J. Neurochem.*, **6**, 117 (1960).
(5) D. R. Curtis and J. C. Watkins, *J. Physiol. (London)*, submitted for publication.
(6) D. R. Curtis, J. W. Phillis and J. C. Watkins, *Brit. J. Pharmacol.*, **16**, 262 (1961).

mammalian and amphibian neurones, than the naturally occurring compounds. It thus became desirable to test a range of N-alkyl derivatives of glutamic and aspartic acid, and, in particular, the separate enantiomers of these compounds, in order to investigate the possibility of stereoselectivity in their actions. The preparation of several new amino acids containing ω-sulfonic and -sulfinic acid groups was also undertaken, in view of the excitatory action of L-cysteic acid. The amino acids synthesized are represented by the general structure shown below, where n = 1, 2 or 3; R = H or CH_3; R′ = H, CH_3, C_2H_5, or n-C_3H_7; X = CO_2H, SO_2H or SO_3H; * = DL, D or L.



I, n = 1, R = H, R′ = CH_3, X = CO_2H; (a) * = DL, (b) * = D, (c) * = L
II, n = 1, R = R′ = CH_3, X = CO_2H; (a) * = DL, (b) * = D
III, n = 1, R = tosyl, R′ = H, X = CO_2H;
* = D and L, (a) R′ = CH_3, * = D and L
IV, n = 2, R = H, R′ = CH_3, X = CO_2H; (a) * = DL, (b) * = D, (c) * = L
V, n = 1, R = H, R′ = C_2H_5, X = CO_2H; (a) * = DL, (b) * = D, (c) * = L
VI, n = 1, R = H, R′ = n-C_3H_7, X = CO_2H; (a) * = D, (b) * = L
VII, n = 1, R = R′ = H, X = SO_3H, * = D
VIII, n = 2, R = R′ = H, X = SO_3H; (a) * = D, (b) * = L
IX, n = 3, R = R′ = H, X = SO_3H, * = DL
X, n = 1, R = H, R′ = CH_3, X = SO_3H; (a) * = DL, (b) * = L
XI, n = 2, R = H, R′ = CH_3, X = SO_3H, * = DL
XII, n = 2, R = R′ = H, X = SO_2H, * = DL

N-Methyl-DL-aspartic acid (Ia) has been prepared previously by the addition of methylamine across the double bond of maleic acid derivatives.[7,8] In the present work the compound was made by the reaction of methylamine with (±)-bromosuccinic acid. The latter reaction can also be used for the synthesis of the separate enantiomers of N-methylaspartic acid (Ib and c),[9,10] but requires the initial preparation of the optically active forms of bromosuccinic acid. In order to avoid resolution of either starting materials or products, N-monomethylation of commercially available D- and L-aspartic acid was investigated as an alternative method for the preparation of the enantiomeric N-methyl derivatives. The reductive condensation of formaldehyde with aspartic acid[11] was unsatisfactory, giving mainly the N,N-dimethyl derivative (II) and only traces of the monomethylated compound, even when a large excess of aspartic acid was present. The method was, however, convenient for the preparation of the

(7) G. Körner and A. Menozzi, *Gazz. chim. ital.*, **19**, 422 (1889).
(8) P. Brookes and J. Walker, *J. Chem. Soc.*, 4409 (1957).
(9) O. Lutz, *Z. physik. Chem.*, **70**, 256 (1910).
(10) O. Lutz and Br. Jirgensons, *Ber.*, **63B**, 448 (1930).
(11) S. Kanao, *J. Pharm. Soc. Japan*, **66**, 4 (1946).

dimethyl derivative. A better, but still unsatisfactory, yield of the monomethyl compound was obtained by the reaction of excess methyl iodide and sodium carbonate with sodium D- or L-aspartate at room temperature for several days, the reaction also yielding succinobetaine. The N-methylaspartic acid enantiomers were preferentially obtained by a three-step procedure which involved the initial preparation of the N-tosyl derivative D- or L-aspartic acid (III), reaction of this derivative with dimethyl sulfate to form the N-methyl-N-tosyl derivative IIIa, and removal of the tosyl group by acid hydrolysis or by reduction with sodium in liquid ammonia. The reductive removal of the tosyl group may have led to some racemization of the product, and the yields were lower than with the hydrolytic method. N-Methyl-D- and -L-glutamic acids (IVb and c) were prepared in a similar way, while the racemic modification was obtained by the reductive condensation of α-ketoglutaric acid with methylamine. The latter compound cyclized to DL-1-methyl-2-pyrrolidone-5-carboxylic acid on heating.

N-Ethyl-DL-aspartic acid (Va) was prepared in moderate yield from (±)-bromosuccinic acid and ethylamine. The N-ethyl and N-*n*-propyl derivatives of D- and L-aspartic acid (Vb, c and VIa, b, respectively) were obtained by the reductive condensation of the appropriate aspartic acid enantiomer (as the monosodium salt) with acetaldehyde and propionaldehyde, respectively.

D-Cysteic acid (VII), D- and L-homocysteic acid (VIIIa and b), and DL-2-amino-5-sulfovaleric acid (IX) were prepared by bromine water oxidation of the appropriate forms of cystine (XIII), homocystine (XIV), and 5,5′-dithiobis(2-aminovaleric acid) (XV), respectively, following a procedure similar to that described by Butz and du Vigneaud[12] for the preparation of DL-homocysteic acid. In order to obtain the DL and L forms of N-methylcysteic acid (Xa and b, respectively), formaldehyde was treated under acidic conditions with DL- and L-cysteine,[13] respectively, to give the corresponding thiazolidine derivatives (XVIa and b), which were then reduced with sodium in liquid ammonia and the resulting N-methyl-DL- and -L-cysteine (XVIIa and b)[14] oxidized by bromine water. A similar preparation of N-methyl-L-cysteic acid has been described recently.[15] N-Methyl-DL-homocysteic acid (XI) was prepared in the same way from the tetrahydro-1,3-thiazine derivative (XVIII), which was obtained

(12) L. W. Butz and V. du Vigneaud, *J. Biol. Chem.*, **99**, 135 (1932).

(13) S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937).

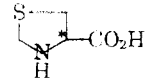
(14) A. H. Cook and J. M. Heilbron in "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson and Sir Robert Robinson, Eds., Princeton University Press, 1949, p. 921.

(15) W. Keller-Schierlein, M. Lj. Mihailović and V. Prelog, *Helv. Chim. Acta*, **42**, 305 (1959).

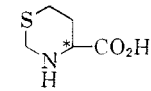
$S(CH_2)_nCH(NH_2)CO_2H$
|
$S(CH_2)_nCH(NH_2)CO_2H$
XIII, $n = 1$
XIV, $n = 2$
XV, $n = 3$

$HSCH_2CHCO_2H$
|
$NHCH_3$
XVII, (a) * = DL
(b) * = L

XIX, RSOSOR
R = $(CH_2)_2CH(NH_2)CO_2H$



XVIa, * = DL
b, * = L



XVIII, * = DL

by the condensation of formaldehyde with DL-homocysteine.

DL-2-Amino-4-sulfinobutyric acid (XII) was prepared by a method based on that described by Lavine[16] for the synthesis of the next lower homolog, cysteinesulfinic acid. Homocystine (XIV) was oxidized with perbenzoic acid to the disulfoxide (XIX), which disproportionated under alkaline conditions into the required sulfinic acid and the starting material, homocystine. The intermediate disulfoxide was not isolated.

Most of the acidic amino acids thus prepared had excitatory actions when tested upon neurones located within the spinal cord and cerebral cortex of the cat[5,17] and spinal cord of the toad.[6] A summary of the pharmacological data is given in Table I. The actions of several of the compounds were considerably stronger than those of glutamic and aspartic acids. The most potent substance was N-methyl-D-aspartic acid, while N-ethyl-D-aspartic acid and D-homocysteic acid also had very strong actions. The corresponding L forms of these compounds had considerably weaker effects, such marked enantiomeric differences being in contrast to the actions of most of the pairs of enantiomers examined previously. Full details of the neuropharmacological results have been reported elsewhere.[5,16,17]

Experimental

Unless otherwise stated, melting points refer to air-dried substances and were determined using an electrically heated copper block. Rotations at 578 and 546 mμ were determined using a Zeiss photoelectric polarimeter on a 1–6% aqueous solutions in a tube of 1 ml. capacity and light path 1 dm.

Ion Exchange and Paper Chromatographic Methods of Isolation and Purification of Products.—Aminoalkane-α,ω-dicarboxylic acids are retained on columns of sulfonated polystyrene resins (H^+ form) but can be eluted by relatively large volumes of water. A routine method of isolation and purification of N-monoalkyl derivatives of these substances, based on the observation that such com-

(16) G. Toennies and J. F. Lavine, *J. Biol. Chem.*, **113**, 571 (1936).
(17) D. R. Curtis and J. C. Watkins, *Nature*, **191**, 1010 (1961).

TABLE I

EXCITATORY POTENCY OF ACIDIC AMINO ACIDS ON NEURONES WITHIN THE CENTRAL NERVOUS SYSTEMS OF THE CAT AND TOAD

Compound	Optical form	Text ref. no.	Relative potency[a]		
			Cat spinal interneurones[b]	Cat cerebral cortex[c]	Toad spinal cord[d]
Aspartic acid	DL				10
	D		7	<7.5	12
	L		10	<7.5	8
Glutamic acid	DL				20
	D		7	<7.5	25–75
	L		10	<7.5	10
Cysteic acid	DL				
	D	VII	7	<7.5	
	L		10	<7.5	6
Homocysteic acid	DL		30	150	250
	D	VIIIa	50	300	
	L	VIIIb	10		
2-Amino-5-sulfovaleric acid	DL	IX	5		
Cysteine sulfinic acid	L		10		
2-Amino-4-sulfinobutyric acid	DL		15		
N-Methylaspartic acid	DL	Ia	40		250
	D	Ib	70	1000	700
	L	Ic	7	10	50
N-Methylglutamic acid	DL	IVa	10		38
	D	IVb	10		
	L	IVc	10		
N-Methylcysteic acid	DL	Xa	5		
	L	Xb	2		
N-Methylhomocysteic acid	DL	XI	5		
N-Ethylaspartic acid	DL	Va	15		
	D	Vb	23		
	L	Vc	2		
N-*n*-Propylaspartic acid	D	VIa	10		
	L	VIb	<1		
N,N-Dimethylaspartic acid	DL	IIa	5		
	D	IIb	2		

[a] The response to a given concentration of a particular substance was taken as a standard, and concentrations of the other substances were varied until the standard response was obtained. The relative potencies determined from these equivalent concentrations have been adjusted to whole numbers based on L-glutamic acid = 10 in the case of cat spinal interneurones[b] and toad spinal cord[d] and on *N*-methyl-D-aspartic acid = 1000 in the case of cat cerebral cortex.[c] [b] Electrophoretic application through micropipets to produce repetitive discharge of

TABLE I, FOOTNOTES (*Continued*)

single cells.[5] [7] Topical application, in Ringer solution, to exposed cerebral cortex of cat, causing spreading depression.[17] [d] Substances added to Ringer solution bathing isolated hemisected spinal cord of the toad, causing a negative potential in the ventral root.[6]

pounds are eluted from the column by water before the unsubstituted acids, was as follows: The mixture to be separated, dissolved in a small volume of water, was allowed to drain into the top of a column of Dowex 50 (or 50 W) (H^+), 200–400 mesh, containing an amount of resin 3–4 times greater than the quantity equivalent to the amino acids, plus a little more than the calculated amount for any other exchangeable cations in the mixture. If the amount of inorganic salt in the mixture was large, the amino acids first were removed from the column with molar aqueous pyridine solution and, after evaporation of the solvent, the recovered amino acid mixture was resorbed on about 4 times the equivalent amount of the resin. In either case, the column was now eluted with water, all fractions being tested with ninhydrin. Samples from fractions containing ninhydrin-reacting material were chromatographed on Whatman No. 1 paper using the system *t*-butanol–acetic acid–water (70:15:15, v./v.) or a buffered mixture containing 2 parts of pyridine–acetic acid–water (30:80:110, v./v. pH 4.1) and 3 parts of 1-butanol. The N-monoalkyl derivatives have higher R_f values than the unsubstituted acids (which were used as markers) and were detected with ninhydrin. Tubes thus shown to contain the required product free from the parent amino acid were combined and evaporated. Aminoalkane-α-carboxylic-ω-sulfonic (and -ω-sulfinic) acids were isolated by sorption on Dowex 1 columns (hydroxide or acetate forms) followed by elution with 6 *N* acetic acid. All fractions were again tested with ninhydrin, but in these cases the products were not greatly contaminated with closely related substances, and the paper chromatographic investigation of fractions was therefore omitted. Fractions containing ninhydrin-reacting material were worked up in groups.

The amino acids were crystallized either from aqueous alcohol or from alcohol–water–ether mixtures. Alcohol (2–3 vol.) was added to an approximately 10% aqueous solution of the amino acid at room temperature or slightly above, and the mixture was cooled to −5°. Alternatively, ether was added at room temperature to the aqueous alcohol solution until the point of incipient turbidity was reached, crystallization then being allowed to proceed at room temperature, or below if necessary. The products frequently contained water of crystallization. Anhydrous compounds were obtained by drying at 80–120° (0.1 mm.) over phosphorus pentoxide, unless otherwise stated.

N-Methyl-DL-aspartic Acid (Ia).—(±)-Bromosuccinic acid (5 g., 0.025 mole) and 35 ml. of 25–30% aqueous methylamine solution were heated together in a sealed tube for 18 hr. at 110°. The mixture was evaporated to dryness under reduced pressure and the resulting gum was dissolved in a small volume of water and allowed to drain into the top of a column containing 70 ml. of Dowex 50 W (H^+). The column was washed first with water until the washings were neutral following prior acidity, then with molar aqueous pyridine solution until tests upon the effluent showed that all ninhydrin-reacting material had been eluted. On evaporation of the pyridine eluate and crystallization of the resulting gum from aqueous alcohol, essentially anhydrous N-methyl-DL-aspartic acid (2.5 g., 67%, m.p. 175°, softening 138°) was obtained. The completely anhydrous product

has been reported[8] to have m.p. 184°. Crystallization from water gave the monohydrate, m.p. 133° (lit.,[8] 134°).

Anal. Calcd. for $C_5H_9NO_4 \cdot H_2O$: C, 36.36; H, 6.71; N, 8.48. Found: C, 36.26; H, 6.63; N, 8.34.

N-Methyl-D-aspartic Acid (Ib) and N-Methyl-L-aspartic Acid (Ic).—A mixture of 1.51 g. (0.0114 mole) of the appropriate isomer of aspartic acid, 2.33 g. (0.022 mole) of *p*-toluenesulfonyl chloride and 17.5 ml. of 8% sodium hydroxide solution was shaken at room temperature until all of the acid chloride had dissolved (4–5 hr.). The solution was made strongly acid with *ca.* 5 ml. of concd. hydrochloric acid and extracted 5 times with 30-ml. volumes of ethyl acetate. The ethyl acetate layers were combined and evaporated under reduced pressure yielding the crude *N*-tosylaspartic acid (2.9 g.) which in most cases crystallized on drying *in vacuo* over sodium hydroxide pellets. To a solution of the crude tosyl derivative in 30 ml. of water containing 2.2 g. of sodium hydroxide was added 3.7 g. (0.029 mole) of dimethyl sulfate, dropwise with stirring during 1 hr. Stirring was continued for a further hr., after which the mixture was acidified and extracted with ethyl acetate as before. The ethyl acetate was evaporated from the combined extracts under reduced pressure, yielding a colorless gum (2.8 g.), which was taken up in 25 ml. of concentrated hydrochloric acid. The solution was heated in a sealed tube for 2 hr., cooled, and evaporated under reduced pressure. The *N*-methylaspartic acid was isolated by ion-exchange chromatography on a column containing 30 ml. of Dowex 50 W (H^+) and crystallized from aqueous alcohol, from which it was obtained as the monohydrate (850 mg., 46%); m.p. 190°. The anhydrous compound was obtained in each case by prolonged drying over phosphorus pentoxide at 85° (0.1 mm.). The L form had $[\alpha]_{578}^{24} + 17.1°$; $[\alpha]_{546}^{24} + 19.2°$ (*c*, 1.40 in water) and the D form $[\alpha]_{578}^{25} - 17.1°$; $[\alpha]_{546}^{25} - 19.5°$ (*c*, 1.49 in water).

Anal. Calcd. for $C_5H_9NO_4 \cdot H_2O$: C, 36.36; H, 6.71; N, 8.48. Found (L form, monohydrate): C, 36.41; H, 6.76; N, 8.47. Found (D form, monohydrate): N, 8.51. Calcd. for $C_5H_9NO_4$: C, 40.81; H, 6.17; N, 9.52. Found (L form, dried sample): C, 40.76; H, 6.14; N, 9.35. Found (D form, dried sample): C, 40.92; H, 6.11; N, 9.32.

Alternative Method (1) for Preparation of N-Methyl-D-aspartic Acid.—D-Aspartic acid (1.8 g., 0.0135 mole) reacted with *p*-toluenesulfonyl chloride, and the crude N-tosyl derivative was methylated with dimethyl sulfate as before. The crude N-methyl-N-tosyl derivative (3.45 g.), obtained by ethyl acetate extraction of the acidified methylation reaction mixture, removal of the ethyl acetate, and drying of the viscous residue under high vacuum, was dissolved in about 100 ml. of liquid ammonia and reduced with 1.5 g. of sodium, which was added in small pieces with stirring over a period of 2 hr. A permanent blue color remained after the last addition and the mixture was decolorized after a further 1 hr. by the addition of a small piece (*ca.* 100 mg.) of Dry Ice. The ammonia was then allowed to evaporate, the last traces being removed *in vacuo* over sulfuric acid. The residue was dissolved in *ca.* 100 ml. of icewater and Dowex 50 (H^+) was added from a supply of 120 ml. of the resin until the supernatant solution was strongly acid and the evolution of carbon dioxide had ceased. The suspension of reaction product and resin was poured onto the top of a column prepared from the remainder of the Dowex 50 (H^+), and the composite column was then washed with water until the pH of the effluent was *ca.* 4. Ninhydrin-reacting material was removed from the column with *ca.* 300 ml. of molar aqueous pyridine solution and,

after its recovery by evaporation of the eluate under reduced pressure, was resubjected to ion-exchange purification through a column containing 25 ml. of Dowex 50 (H^+), with elution by water in the usual way: yield 500 mg. (22%); m.p. 188°; $[\alpha]_{578}^{22} - 16.1°$; $[\alpha]_{546}^{22} - 18.5°$ (c, 1.27 in water).

Anal. Calcd. for $C_5H_9NO_4$: C, 40.81; H, 6.17; N, 9.52. Found (sample dried *in vacuo*): C, 40.96; H, 6.25; N, 9.45.

Alternative Method (2) for Preparation of N-Methyl-D-aspartic Acid, and Isolation of D-Succinobetaine.—A mixture of 1.33 g. (0.01 mole) of D-aspartic acid, 2.12 g. (0.02 mole) of sodium carbonate, 2.84 g. (0.02 mole) of methyl iodide and 10 ml. of water was stirred for 9 days at room temperature. The solution was filtered and to the filtrate was added *ca.* 20 ml. of Dowex 50 (H^+). The suspension of the reaction mixture and resin then was poured gently on the top of a column containing 30 ml. of Dowex 50 (H^+) and water was passed through the composite column. The first acidic eluates gave a negative reaction with ninhydrin and contained D-succinobetaine. The ninhydrin-positive fractions which followed contained successively N-methyl-D-aspartic acid and unchanged starting material. Fractions containing only the monomethylated substance, as demonstrated by paper chromatography, were evaporated and yielded 250 mg. (15%) of the relatively pure monohydrate, m.p. 187–188°, $[\alpha]_{578}^{19} - 16.3$ (c, 1.0 in water), after one recrystallization from a water–alcohol–ether mixture. D-Succinobetaine (m.p. 149°) was isolated in about 10% yield from the first acidic eluates obtained in another experiment.

Anal. Calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00. Found: C, 47.77; H, 7.59; N, 7.86.

N-Methyl-DL-glutamic Acid (IVa).—A solution of 3 g. of α-ketoglutaric acid and 100 ml. of 25–30% aqueous methylamine solution was hydrogenated at 80° and 75 kg./cm.² for 12 hr. in the presence of Raney nickel. The solution was filtered and the filtrate evaporated under reduced pressure. The crude product, containing a mixture of N-methyl-DL-glutamic acid and DL-1-methyl-2-pyrrolidone-5-carboxylic acid, was taken up in 30 ml. of concd. hydrochloric acid and heated in a sealed tube at 110° for 3 hr. The solution was evaporated under reduced pressure, and the resultant gum was taken up in *ca.* 10 ml. of water for application to the top of a column containing 50 ml. of Dowex 50 W (H^+). After washing the column with *ca.* 200 ml. of water, the product was eluted with 150 ml. of molar aqueous pyridine solution. Evaporation of the pyridine eluate under reduced pressure and crystallization of the residue from a water–alcohol–ether mixture (charcoal) yielded 1.34 g. (40%) of N-methyl-DL-glutamic acid, m.p. 157° (lit.,[18] 156–158°).

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.56; H, 7.02; N, 8.51.

It should be noted that small amounts of DL-glutamic acid sometimes are formed during the reaction, presumably because of contamination of commercial methylamine solutions with ammonia. The presence of glutamic acid in the product can be detected by paper chromatography and it may be separated from the methylated compound by ion-exchange chromatography on Dowex 50 (H^+) with elution by water in the usual way.

Cyclization of N-Methyl-DL-glutamic Acid.—On drying a sample of N-methyl-DL-glutamic acid at 85° *in vacuo* for several days, partial loss of water occurred. Complete conversion into the cyclic product, DL-1-methyl-2-pyrrolidone-5-car-

(18) S. Sugasawa, *J. Pharm. Soc. Japan*, **No. 550**, 1041 (1927).

boxylic acid, was effected by heating the open chain compound *in vacuo* at its melting point for 1 hr. and then allowing the molten sample to resolidify.

Anal. Calcd. for $C_6H_9NO_3$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.20; H, 6.41; N, 9.93. The cyclic compound has the same m.p. (157°) as the starting material, suggesting that the latter is converted into the former during melting point determinations.

N-Methyl-D-glutamic Acid (IVb) and N-Methyl-L-glutamic Acid (IVc).—These enantiomers were made following the alternative method (1) for preparation of N-methyl-D-aspartic acid. To avoid cyclization, the samples were dried for prolonged periods *in vacuo* over phosphorus pentoxide at room temperature. The L form had m.p. 146° (from water–alcohol–ether) and $[\alpha]_{578}^{19} + 21.0°$; $[\alpha]_{546}^{19} + 23.9°$ (*c*, 2.59 in water). The D form had m.p. 147° (from water–alcohol–ether) and $[\alpha]_{578}^{20} - 21.5°$; $[\alpha]_{546}^{20} - 24.8°$ (*c*, 2.18 in water).

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found (L form): C, 44.66; H, 7.03; N, 8.60. Found (D form): C, 44.61; H, 7.07; N, 8.75.

N-Ethyl-DL-aspartic Acid (Va).—This compound was prepared from 5 g. (0.025 mole) of (±)-bromosuccinic acid and 35 ml. of 33% aqueous ethylamine solution in an analogous way to N-methyl-DL-aspartic acid; yield 2.2 g. (54%), m.p. 180° (from water–alcohol–ether).

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found (sample dried *in vacuo*): C, 44.72; H, 6.93; N, 8.71.

N-Ethyl-D-aspartic Acid (Vb) and N-Ethyl-L-aspartic Acid (Vc).—A solution of 2 g. (0.015 mole) of the appropriate isomer of aspartic acid in 30 ml. of 0.5 *N* sodium hydroxide solution was shaken with hydrogen in the presence of platinum oxide (50 mg.) while a solution of 0.72 g. (0.016 mole) of acetaldehyde in 10 ml. of water was added portionwise under hydrogen during 5 hr. The mixture was shaken with hydrogen for a further 20 hr., filtered, and the filtrate evaporated under reduced pressure. The resulting gum was subjected to ion-exchange purification on a column containing 60 ml. of Dowex 50 (H^+) in the routine manner. The products crystallized from water–alcohol–ether mixtures as the monohydrates, m.p. 181° in each case; yield 1.05 g. (37%). The anhydrous compounds were obtained on drying *in vacuo* over phosphorus pentoxide at 85°. The L form had $[\alpha]_{578}^{20} + 18.5°$; $[\alpha]_{546}^{20} + 21.1°$ (*c*, 5.31 in water) and the D form $[\alpha]_{578}^{20} - 18.1°$; $[\alpha]_{546}^{20} - 20.6°$ (*c*, 6.03 in water).

Anal. Calcd. for $C_6H_{11}NO_4 \cdot H_2O$: C, 40.22; H, 7.31; N, 7.82. Found (L form, monohydrate): C, 40.23; H, 7.26; N, 7.70. Found (D form, monohydrate): C, 40.49; H, 7.31; N, 7.70. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found (L form, dried sample): C, 44.89; H, 6.64; N, 8.63. Found (D form, dried sample): C, 44.86; H, 6.90; N, 8.56.

N-*n*-Propyl-D-aspartic Acid (VIa) and N-*n*-Propyl-L-aspartic Acid (VIb).—These compounds were prepared from 2 g. (0.015 mole) of the appropriate isomer of aspartic acid and 1.9 g. (0.033 mole) of propionaldehyde by the method described for N-ethyl-D- and -L-aspartic acids. Unlike the N-ethyl derivatives the compounds crystallized without water of crystallization from water–alcohol–ether mixtures; yield 1.3 g. (49%); m.p. 172°. The L form had $[\alpha]_{578}^{25} + 12.3°$; $[\alpha]_{546}^{25} + 14.1°$ (*c*, 2.77 in water) and the D form $[\alpha]_{578}^{23} - 12.2°$; $[\alpha]_{546}^{23} - 13.9°$ (*c*, 2.13 in water).

Anal. Calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00. Found (L form): C, 48.12; H, 7.55; N, 8.02. Found (D form): C, 48.06; H, 7.62; N, 7.79.

N,N-Dimethyl-DL-aspartic Acid (IIa) and N,N-Dimethyl-D-aspartic Acid

(**IIb**).—To a solution of 2 g. (0.015 mole) of DL- or D-aspartic acid in 75 ml. of 0.2 *N* sodium hydroxide solution was added 8 ml. of 40% formaldehyde solution and 500 mg. of 10% palladium on charcoal. The mixture was shaken with hydrogen until the theoretical amount had been absorbed, after which it was filtered, and the filtrate concentrated. Sodium ion and small amounts of aspartic acid and N-methylaspartic acid were removed on a column of Dowex 50 (H^+) in the usual way, the ninhydrin-positive fractions in this case being rejected, while the preceding acidic, but ninhydrin-negative, eluates were combined and evaporated. The product was crystallized from a water–alcohol–ether mixture in each case. The DL form had m.p. 188°, and the D form had m.p. 182°, $[\alpha]_{578}^{19}$ − 14.7°, $[\alpha]_{546}^{19}$ − 16.7° (*c*, 2.52 in water); yield 1.8 g. (74%).

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found (DL form, dried *in vacuo*): N, 8.49, 8.61. Found (D form, dried *in vacuo*): C, 44.94; H, 6.81; N, 8.57. For the L isomer, Kanao[11] gives m.p. 196°; $[\alpha]^{20}_D$ + 15.84°, and Bowman and Stroud[19] give m.p. 184°, $[\alpha]^{18}_D$ + 14.4°; $[\alpha]_{546}^{14.4}$ + 17.5° (c, 3.06 in water).

D-Cysteic Acid (VII).—Bromine was added dropwise with shaking to a suspension of 220 mg of D-cystine in 5 ml. of water until the initially transient yellow color persisted for 1 hr. The solution was evaporated under reduced pressure, and final traces of hydrogen bromide were removed by repeated trituration of the residue with small quantities of cold alcohol. That part of the product which dissolved in the alcohol was precipitated from the washings by the addition of ether and recovered by filtration, and the combined product (near quantitative yield) was recrystallized from a water–alcohol–ether mixture, from which it separated as the monohydrate (205 mg.), m.p. 245° (dec.); $[\alpha]_{578}^{20}$ − 6.1°; $[\alpha]_{546}^{20}$ − 7.3° (*c*, 1.57 in water). Cavallini, De Marco and Mondovì[20] give $[\alpha]^{20}_D$ − 6.9° (*c*, 3.0 in water).

Anal. Calcd. for $C_3H_7NO_5S \cdot H_2O$: C, 19.25; H, 4.85. Found (monohydrate): C, 19.11; H, 4.78. Calcd. for $C_3H_7NO_5S$: C, 21.30; H, 4.17; N, 8.28. Found (sample dried *in vacuo*): C, 21.28; H, 4.13; N, 8.09.

D-(VIIIa) and L-(VIIIb) Homocysteic Acids.—These substances were prepared from D- and L-homocystine, respectively, by the method described for D-cysteic acid. The products crystallized from water–alcohol–ether mixtures without solvent of crystallization. The L form had m.p. 261° dec.; $[\alpha]_{578}^{19}$ + 17.0°; $[\alpha]_{546}^{19}$ + 19.7° (*c*, 0.57 in water). The D form had m.p. 267° dec.; $[\alpha]_{578}^{21}$ − 18.0°; $[\alpha]_{546}^{21}$ − 21.0° (*c*, 1.305 in water).

Anal. Calcd. for $C_4H_9NO_5S$: C, 26.22; H, 4.95; N, 7.65; S, 17.50. Found (L form): C, 26.11; H, 4.89; N, 7.64; S, 17.62. Found (D form): C, 26.27; H, 5.16; N, 7.54; S, 17.88.

DL-2-Amino-5-sulfovaleric Acid (IX).—A suspension of 500 mg. of 5,5′-dithiobis(2-aminovaleric acid)[21] in 20 ml. of water was oxidized with bromine as described for the other sulfonic acids. The product was crystallized from a water–alcohol–ether mixture; yield 600 mg. (90%), m.p. 274° dec.

Anal. Calcd. for $C_5H_{11}NO_5S$: C, 30.44; H, 5.62; N, 7.10; S, 16.26. Found (sample dried *in vacuo*): C, 30.29; H, 5.78; N, 7.07; S, 16.26.

(±)-Tetrahydro(1,3-thiazine)-4-carboxylic Acid (XVIII).—Two grams (0.0075

(19) R. E. Bowman and H. H. Stroud, *J. Chem. Soc.*, 1342 (1950).
(20) D. Cavallini, C. De Marco and B. Mondovì, *J. Biol. Chem.*, **230**, 25 (1958).
(21) V. du Vigneaud, H. M. Dyer, C. B. Jones and W. I. Patterson, *ibid.* **106**, 401 (1934).

mole) of a mixture of DL- and *meso*-homocystine was dissolved in 50 ml. of 4 *N* hydrochloric acid and 4 g. (0.034 g.-atom) of tin foil, cut into small pieces, was added. The mixture was left at room temperature overnight, and hydrogen sulfide was then passed in until precipitation of stannous sulfide was complete. The suspension was filtered and hydrogen sulfide was removed from the filtrate with a stream of nitrogen. After evaporation of the mixture at 40° (20 mm.), the residue was dissolved in 10 ml. of water. To this solution was added 1.4 ml. of 40% aqueous formaldehyde solution and the mixture was allowed to stand overnight. Pyridine (1.7 ml.) was then added, and the resulting precipitate (0.43 g.) was removed by filtration. The filtrate was diluted with an equal volume of alcohol and from the solution (±)-tetrahydro(1,3-thiazine)-4-carboxylic acid monohydrate (0.8 g.), m.p. 208–209°, crystallized on standing. A further 0.4 g. (m.p. 207°) was obtained from the mother liquor after the addition of more alcohol.

Anal. Calcd. for $C_5H_{11}NO_3S$: C, 36.36; H, 6.71; N, 8.48; S, 19.41. Found: C, 36.47; H, 6.74; N, 8.43; S, 19.17.

N-Methyl-DL-homocysteic Acid (XI).—(±)-Tetrahydro(1,3-thiazine)-4-carboxylic acid monohydrate (1.2 g., 0.007 mole) was dissolved in 100 ml. of liquid ammonia and small pieces of sodium (total 0.5 g.) were added with stirring over 1 hr. After the last addition of sodium, the blue color was discharged by the addition of a small piece of Dry Ice (*ca.* 100 mg.) and the ammonia was allowed to evaporate. Final traces of ammonia were drawn off at the water pump and the residue was dissolved in a little water. The mixture was made strongly acid with hydrobromic acid and evaporated under reduced pressure. The solid residue was triturated with 50 ml. of alcohol, filtered, and the filter cake washed with more alcohol. The filtrate and washings were combined and evaporated under reduced pressure yielding 3 g. of a solid mixture of inorganic salts and N-methyl-DL-homocysteine hydrobromide. Bromine was added dropwise with stirring to a solution of this mixture in 30 ml. of water until the transient yellow color produced by a drop of bromine persisted for 1 hr. After evaporation of the acidic solution and removal of most of the residual hydrogen bromide by repeated washing with an ether–alcohol mixture (4:1, v./v.), the product was freed of alcohol and ether on the water pump, dissolved in a little water and the solution allowed to drain into the top of a column containing 25 ml. of Dowex 1 (OH^- form). The column was washed with water until the effluent was neutral and the product was eluted with 6 *N* acetic acid, 50 ml. fractions being collected. Each of the eleven fractions collected immediately after the effluent became acid gave, on evaporation under reduced pressure, crystalline or semi-crystalline residues, which were recrystallized separately from water–alcohol–ether mixtures to yield a total of 300 mg. of N-methyl-DL-homocysteic acid, m.p. 265° dec.

Anal. Calcd. for $C_5H_{11}NO_5S$: C, 30.46; H, 5.62; N, 7.10; S, 16.26. Found (sample dried *in vacuo*): C, 30.35; H, 5.67; N, 7.08; S, 16.26.

N-Methyl-DL- (Xa) and N-Methyl-L-cysteic Acid (Xb).—The DL and L forms of 4-thiazolidinecarboxylic acid were prepared by the method of Ratner and Clarke.[13] The appropriate form of 4-thiazolidinecarboxylic acid monohydrate (800 mg.) was dissolved in 30 ml. of liquid ammonia and small pieces of sodium (0.30 g. in all) were added with stirring until the solution became permanently blue. The color was discharged with a small piece of solid carbon dioxide and the ammonia was allowed to evaporate, final traces being drawn off at the water pump. The residue was taken up in a little water and brought to pH 4–5 with hydrobromic acid. The precipitate (250 mg.) of a dimeric by-product[14] (DL

form, m.p. 226°; L form, m.p. 244°) was filtered off and the filtrate was made strongly acid with hydrobromic acid and evaporated under reduced pressure. The resulting gum was extracted with alcohol, the residue obtained on removal of the alcohol was treated with bromine, and the N-methylcysteic acid was isolated by ion exchange chromatography on Dowex 1 (OH^-) with elution by 6 *N* acetic acid solution exactly as described above for the higher homolog. The product (450 mg.) crystallized from a water–alcohol–ether mixture. The DL form had m.p. 233° dec. The L form had m.p. 235° dec., $[\alpha]_{578}^{19} + 4.1°$; $[\alpha]_{546}^{19} + 4.8°$ (*c*, 2.8 in water); Keller–Schierlein, *et al.*,[15] give m.p. 228–229° dec., and $[\alpha]_D + 4.26°$ (*c*, 5.216 in water).

Anal. Calcd. for $C_4H_9NO_5S \cdot H_2O$: C, 23.88; H, 5.51. Found (L form, monohydrate): C, 24.05; H, 5.52. Calcd. for $C_4H_9NO_5S$: C, 26.22; H, 4.95; N, 7.65; S, 17.50. Found (DL form, dried sample): C, 26.37; H, 4.98; N, 7.61; S, 17.49. Found (L form, dried sample): C, 26.11; H, 4.98; N, 7.59; S, 17.69.

DL-2-Amino-4-sulfinobutyric Acid (XII).—The method used for the preparation of this substance was modeled on that described by Lavine[16] for the synthesis of L-cysteinesulfinic acid. A mixture of the DL and *meso* forms of homocystine (1.34 g.) was added to 25 ml. of cold (0°) purified acetonitrile[22] in a 100-ml. volumetric flask. The exactly equivalent quantity of 62% perchloric acid then was added with cooling, followed by a solution of acetic anhydride (sufficient to react with 90% of the water added with the perchloric acid) in 30 ml. of acetonitrile. The mixture was filtered to remove a few mg. of undissolved homocystine, and a solution of 1.66 g. of perbenzoic acid[23] in 15 ml. of chloroform was added to the filtrate, which was then made up to 100 ml. with acetonitrile. The solution was allowed to stand at room temperature for 1 hr., during which time a crystalline deposit formed. The mixture was extracted twice with 25-ml. volumes of *N* hydrochloric acid and the aqueous layers were combined and extracted with chloroform (4 × 5 ml.). The aqueous layer was brought to pH 4 with 8 *N* ammonium hydroxide solution and allowed to stand at 0° overnight. In contrast to the preparation of L-cysteinesulfinic acid, no precipitate formed at this stage. The solution was allowed to warm to room temperature and made strongly alkaline with concentrated ammonia solution. After 1 hr. at room temperature, the mixture was evaporated (temp. < 40°) under reduced pressure. The residue was extracted with 30 ml. of water and filtered to remove insoluble material (440 mg. m.p. > 300°), which was probably homocystine. The filtrate was evaporated under reduced pressure yielding 5.9 g. of a solid mixture of inorganic salts and the desired product. A solution of this mixture in 50 ml. of water was allowed to drain into the top of a column containing 100 ml. of Dowex 1 (acetate), and the column was washed with water until the washings were neutral. The sulfinic acid was then eluted with 6 *N* acetic acid, the eluate being collected in 250 ml. fractions. Ninhydrin-reacting material appeared in the 750–1500 ml. range. These fractions were worked up individually, yielding DL-2-amino-4-sulfinobutyric acid (430 mg.) as colorless needles from a water–alcohol–ether mixture; m.p. 178° dec.

Anal. Calcd. for $C_4H_9NO_4S$: C, 28.75; H, 5.43; N, 8.38; S, 19.18. Found (sample dried *in vacuo*): C, 28.74; H, 5.44; N, 8.30; S, 19.23.

(22) Dried first over K_2CO_3 then refluxed over P_2O_5 and fractionated [G. Toennies and J. F. Lavine, *J. Biol. Chem.*, **100**, 463 (1933)].

(23) "Organic Syntheses," Coll. Vol. I, 2nd Edition, H. Gilman and A. H. Blatt, Eds., John Wiley and Sons, New York, N. Y., 1948, p. 431.

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New Psychotropic Agents.[1] II. Derivatives of 5,6-Dihydrodibenz[b,e]azepine (5,6-Dihydromorphanthridine)

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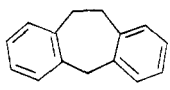
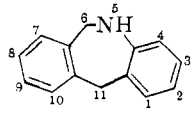
Sodium borohydride treatment of 6,11-diketo-5,6-dihydrodibenz[b,e]azepine produced 11-hydroxy-6-keto-5,6-dihydrodibenz[b,e]azepine. Alkylation with 3-dimethylaminopropylmagnesium chloride also gave an 11-hydroxy derivative, 11-(3-dimethylaminopropyl)-11-hydroxy-6-keto-5,6-dihydrodibenz[b,e]azepine. This latter compound was used as an intermediate for the synthesis of other 11-substituted dibenz[b,e]azepines. The pharmacological activities of some of these compounds are discussed briefly.

In the course of an investigation of the pharmacological properties of compounds containing tricyclic ring systems analogous to the phenothiazine ring, it was found that certain derivatives of dibenzo[a,d][1,4]cycloheptadiene (I) exhibited a number of interesting effects on the central nervous system.[1]



I



II

One of these compounds, namely, 5-(3-dimethylaminopropylidene)-dibenzo[a,d][1,4]cycloheptadiene, has since found clinical usage as an antidepressant agent.[4] It was of interest to investigate similar derivatives of ring systems structurally related to I. The present paper describes a part of this study, the synthesis of 5,6-dihydrodi-

(1) For the first paper in this series, see S. O. Winthrop, M. A. Davis, G. S. Myers, J. G. Gavin, R. Thomas, and R. Barber, *J. Org. Chem.*, **27**, 230 (1962).

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(4) (a) F. J. Ayd, Jr., *Psychosomatics*, **I**, 320 (1960). (b) W. Dorfman, *Psychosomatics*, **I**, 153 (1960). (c) H. Freed, *Am. J. Psychiat.*, **117**, 455 (1960).