The crude solid residue was recrystallized from methanol–ether and gave 49 g. (56%) of product, m.p. 234–237°.

Anal. Calcd. for $C_6H_{15}ClN_2O$: C, 43.5; H, 9.0; Cl, 21.3; N, 16.8. Found: C, 43.4; H, 9.0; Cl, 21.0; N, 16.4.

α-Bromoamides.—The amides listed in Table I were made by reaction of acid chlorides with ammonia or amines either in aqueous or in anhydrous media. If a concentrated aqueous solution of the base was available commercially, the aqueous procedure was used. Ether or chloroform generally was used for the anhydrous bases. After filtration of the hydrochlorides of the bases the products were obtained by evaporation of the solvent and purified in the manner indicated in Table I.

2-Ethyl-2-(2-bromo-2-ethylbutyramido)-butyramide (LV).—To an ice-cold, stirred solution of 35 g. (0.21 mole) of 2-amino-2-ethylbutyramide hydrochloride in 300 cc. of water and 50 cc. of acetone there was added, simultaneously, during 1 hour, a solution of 47.1 g. (0.22 mole) of 2-bromo-2-ethylbutyryl chloride in 100 cc. of acetone and 490 cc. (0.49 mole) of 1 *N* sodium hydroxide solution. The product separated as a white solid which was filtered and air-dried.

N-(2-Bromo-2-ethylbutyryl)-glycine (LII).—To a solution of 2.8 g. (0.01 mole) of N-(2-bromo-2-ethylbutyryl)-glycine ethyl ester[23] in 25 cc. of methanol, there was added, dropwise, at 5°, with stirring, 2 cc. (0.01 mole) of 5 *N* sodium hydroxide solution. After having been stored at room temperature overnight the solution was concentrated under reduced pressure to remove most of the methanol and the resulting solution was acidified with dilute hydrochloric acid to *p*H 1. The product separated from the cooled solution as a white precipitate which was filtered, washed with water, and dried.

1-(2-Bromo-2-ethylbutyryl)-3-methylurea (LVII).—A mixture of 25 g. (0.34 mole) of N-methylurea and 36.1 g. (0.12 mole) of 2-bromo-2-ethylbutyryl chloride was heated on a steam-bath for six hours with occasional swirling. A clear reddish-brown solution formed to which was added 100 g. of ice and 50 cc. of water. A gum formed which solidified upon trituration. The solid was filtered and washed first with cold water and then with petroleum ether (b.p. 20–40°). The crude solid was extracted with petroleum ether in a Soxhlet apparatus for 4 hours. Concentration of the solution to a small volume gave the product as a white solid.

1-(2-Bromo-2-methylpropionyl)-3-methylurea (XXIII).—A mixture of 20 g. (0.12 mole) of 2-bromo-2-methylpropionyl chloride and 17.8 g. (0.24 mole) of methylurea was heated on a steam-bath for 0.5 hour. An oily solid formed and the mass was treated with water and filtered. The white solid was dried on a porous plate.

1-(2-Bromo-2-methylbutyryl)-3-methylurea (XXX).—A mixture of 37.1 g. (0.5 mole) of dried methylurea and 39.9 g. (0.2 mole) of 2-bromo-2-methylbutyryl chloride was stirred and heated for 2 hours in an oil-bath at 60–87°. To the mixture, consisting of an amber liquid containing some colorless crystals, was added 100 cc. of water. On stirring and cooling, colorless crystals separated. These were filtered, ground in a mortar with ice-water, and filtered again.

PEARL RIVER, N. Y.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN CO.,[a] AND THE UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL[b]]

The Behavior of the Isomers of α,ε-Diaminopimelic Acid on Paper Chromatograms

BY LIONEL E. RHULAND,[a] ELIZABETH WORK,[b] R. F. DENMAN[b] AND D. S. HOARE[b]

RECEIVED MAY 2, 1955

The amino acid α,ε-diaminopimelic acid can be resolved into the DD- and LL-isomers by means of paper chromatography without resorting to the use of optically active solvents. A solvent system is described in which the isomeric forms of α,ε-diaminopimelic acid can be examined in a complex protein hydrolysate.

Work[1] isolated α,ε-diaminopimelic acid (DAP) from *Corynebacterium diphtheriae* and suggested that it was the *meso*-isomer. Comparative paper chromatographic studies of this material with synthetic DAP[1,2] in a series of ten solvent systems indicated that the synthetic and natural DAP preparations behaved the same, although some streaking of the synthetic material was noted in phenol (NH_3 atmos.) using Whatman No. 4 paper.[3]

In the course of routine chromatographic studies with six solvent systems differing from those of Work[1] or Wright and Cresson,[2] we observed the presence of only one ninhydrin-positive material in synthetic DAP[4] (*cf.* Table I). However, in a methanol (80)–water (20)–pyridine (4) system,[5] we observed that this material could be separated into two distinct components. Two-dimensional paper chromatography in the above system (*cf.* Fig. 1) demonstrated that the separation was not due to the formation of ionic species or solvent artifacts and that both components were biologically active for the DAP auxotroph of *E. coli* 173–25.[6]

TABLE I

THE CHROMATOGRAPHIC BEHAVIOR OF SYNTHETIC DIAMINOPIMELIC ACID IN A VARIETY OF SOLVENT SYSTEMS

Whatman No. 1 paper, descending system, temp. 25°, 20 mcg., α,ε-diaminopimelic acid. Ninhydrin used for developing chromatogram.

Solvent system	*Rf*
n-BuOH (81)–H_2O (19)–$PTSO_3H$ (0.25)[a]	0.16
H_2O (19)–*n*-BuOH (81)	0
H_2O (90)–*n*-BuOH (4)–$PTSO_3H$ (0.25)	0.8
n-BuOH (4)–H_2O (96)	0.8
Methanol (80)–H_2O (20)–pyridine (4)	0.17, 0.26[b]
n-BuOH (50)–H_2O (25)–acetic acid (25)	0.21

[a] *p*-Toluenesulfonic acid. [b] Two components.

These data suggested that isomeric forms of DAP were being separated by means of paper chromatography. Confirmation of this fact was obtained by chromatography of the resolved isomers of DAP (LL, DD and *meso*)[7]; the LL- and DD-isomers were re-

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(2) L. D. Wright and E. L. Cresson, *Proc. Soc. Exptl. Biol. Med.*, **82**, 354 (1953).

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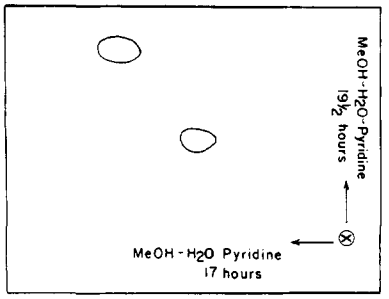

Fig. 1.—Separation of synthetic diaminopimelic acid (20 μg.) into two biologically active components by a methanol (80)–water (20)–pyridine (4) system. After 2-dimensional chromatography, strip assayed on bioautographic plate using DAP auxotroph of *Escherichia coli* 173-25 (kindly supplied by Dr. B. D. Davis).

solved from one another, whereas the DD- and *meso*-isomers were not separated (*cf.* Fig. 2a).

With the object of applying this chromatographic technique to the study of the metabolism of DAP isomers in microörganisms, conditions were varied so as to obtain the best separation of the isomers from each other and from other naturally occurring amino acids, particularly lysine, a metabolic product of DAP. Whatman No. 1 paper produced better resolution than No. 4, and was used in all the work. A variety of basic solvents were investigated (*cf.* Table II, Fig. 2). In some, all three isomers of DAP behaved identically, but in ethanol/ammonia and aqueous phenol (ammonia atmosphere), the LL-isomer moved faster than the other two isomers. However, neither of these solvents produced satisfactory resolution overnight, and there was considerable overlapping of DAP with other amino acids. Alterations in the proportion of pyridine in Redfield's solvent system between 4 and 20 parts had no adverse effect on resolving power; the R_f values of all the DAP isomers were decreased equally and the positions relative to the other amino acids were changed slightly. In no case could lysine be separated from all the isomers, and there was always some streaking, particularly of the *meso*-isomer.

TABLE II

BEHAVIOR OF DAP ISOMERS AND LYSINE IN BASIC SOLVENTS

Solvent system	Dist. run by *meso*-DAP in 17 hr. (cm.)	*R* *meso* DAP LL-DAP	*R* *meso* DAP DD DAP[a]	Lysine
Methanol (77)–H_2O (20)–pyridine (10)	12	1.4	1	1.4
Methanol (80)–H_2O (17.5)–10 *N*-HCl (2.5)–pyridine (10)	17	1.3	1	1.9
Aqueous phenol (NH_3 atmos.)	4	1.3	1	2.5
Ethanol (77)–2 *N* NH_3 (23)	8	1.3	1	2.1
Amyl alcohol (35)–pyridine (35)–H_2O (30)	2	1	1	..
t-Butanol (40)–methyl ethyl ketone (40)–H_2O (20)-diethylamine (4)	13	1	1	..
n-Butanol (40)–ethanol (10)–H_2O (49)–ammonia (0.880) (1)	14	1	1	..
Lutidine 2:4/2:5 (2)–H_2O (1)	1	1	1	1

[a] Distance run by amino acid/distance run by *meso*-DAP.

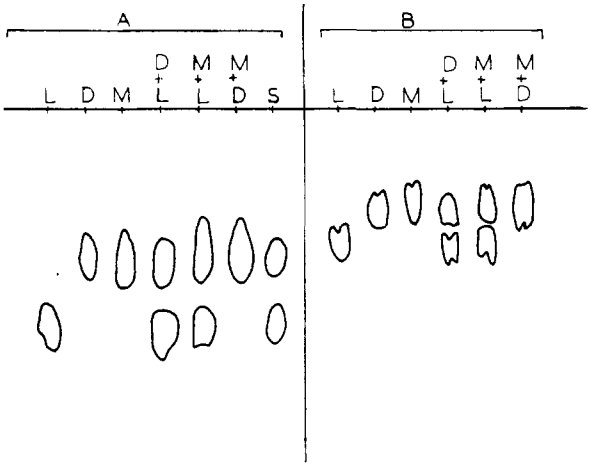

Fig. 2.—Chromatogram of diaminopimelic acid isomers in (a) methanol (77)–water (20)–pyridine (10) and (b) aqueous phenol–(NH_3 atmosphere), run 26 hr. S denotes synthetic DAP, M, D and L denote *meso*-, DD- and LL-DAP, respectively.

The solvent mixture methanol (77)–water (20)–pyridine (10) was found to be most satisfactory for separating the DAP isomers overnight in the absence of basic amino acids. Addition of HCl to the mixture, while having no adverse effect on resolution, rendered the solvent more suitable for the chromatography of complex amino acid mixtures, as overlap with lysine and streaking were both eliminated.

The mixture finally chosen for routine chromatographic examination of the isomeric forms of DAP in biological materials, such as enzymic reaction mixtures or protein hydrolysates, contained methanol (80)–water (17.5)–10 *N* HCl (2.5)–pyridine (10). With the exception of cysteic acid, the oxidation product of cystine, all the naturally occurring amino acids travel faster than the DAP isomers, and are separated from DAP on a single-dimensional chromatogram run overnight (*cf.* Fig. 3). A chromatogram of an acid hydrolysate of *Mycobacterium tuberculosis*, which contains *meso*-DAP[1,8] is illustrated in Fig. 3 at the spot marked "Hyd."

The presence of both HCl and pyridine in the solvent causes the DAP spots to give an unusual ninhydrin color reaction. Chromatograms were developed by dipping in ninhydrin in acetone (0.1% w./v.) and heating for 2 min. at 100°. The common amino acids appeared as characteristic purple, blue, or yellow spots which faded very rapidly; the DAP spots were bright olive green and on standing changed to a stable yellow color with a faint pink fluorescence in ultraviolet light. This color reaction was not given by DAP diamide or by other straight-chain diaminodicarboxylic acids and appeared to be specific for α,ε-diaminopimelic acid, its monoamide and its homolog which occurs in certain Actinomycetes.[9] It is a useful test to differentiate between DAP and cysteic acid.

The resolution of the LL- and DD-isomers of DAP is achieved by three different solvent systems all devoid of any asymmetric components, but which

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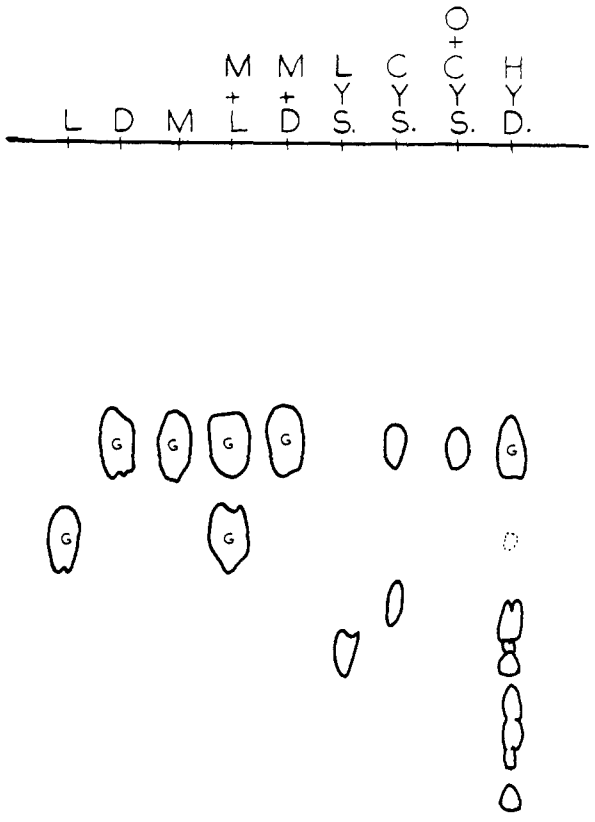

Fig. 3.—Descending chromatogram in methanol (80)–water (17.5)–10 *N* HCl (2.5)–pyridine (10). Abbreviations are as follows: Lys = L-lysine, cys = cystine, O + cys = H_2O_2 + cystine, hyd. = acid hydrolysate of *Mycobacterium tuberculosis* equiv. to 2 mg. dry wt. of organism, L, M, D as in Fig. 2, G = green ninhydrin color.

are all basic. The effect is evidently specific to DAP since a number of other synthetic amino acids, including the DAP homologs α,δ-diaminoadipic acid and α,ζ-diaminosuberic acid, could not be resolved into separate components on the methanol–water pyridine chromatograms (*cf.* Table III). This suggests that the cationic forms of DAP are selectively and differentially absorbed by some asymmetric element in the paper, resulting in a different partition of the LL-isomer in certain solvents. Kotake[10] reported that DL-glutamic acid could be resolved in a solvent system containing the optically active *l*-methyl-(β-phenylisopropyl)-amine. To our knowledge, however, the resolution of stereoisomers of an aliphatic amino acid without the use of optically active solvents is a new observation. Certain aromatic amino acids have been resolved in this way.[11]

TABLE III

THE CHROMATOGRAPHIC BEHAVIOR OF A SERIES OF SYNTHETIC ALIPHATIC AMINO ACIDS IN A METHANOL (80)–WATER (20)–PYRIDINE (4) SYSTEM

Amino acid	*Rf*
α,δ-Diaminoadipic acid	0.23
α,ζ-Diaminosuberic acid[a]	.31
α-Aminopimelic acid	.65
ε-C-Methyllysine[b]	.33
α,γ-Diaminoheptanoic acid[c] (streaked)	.55
α,γ-Diamino-β-methylbutyric acid[c]	.35
α-Methylglutamic acid	.62
α-Amino-β-methylpimelic acid	.62

[a] Kindly supplied by Dr. D. Simmonds, Medical Research Institute, London. [b] Kindly supplied by Dr. A. D. McLaren, University of California. [c] Kindly supplied by Dr. H. E. Carter, University of Illinois.

(10) M. Kotake, T. Sakan, N. Nakamura and S. Senole, THIS JOURNAL, **73**, 2973 (1951).

(11) C. E. Dalgliesh, *J. Chem. Soc.*, 3940 (1952).

THE RESEARCH LABORATORIES
THE UPJOHN COMPANY
KALAMAZOO, AND
UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
LONDON, W. C. 1, ENGLAND

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BOSTON UNIVERSITY]

A New Synthesis of Phloionic Acid[1]

BY WALTER J. GENSLER AND HERBERT N. SCHLEIN

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A synthesis of 9,10-dihydroxyoctadecanedioic acid (phloionic acid) is reported. The sodium derivative of 8-chloro-1-octyne couples with 6-iodo-1-chlorohexane to give 1,14-dichloro-7-tetradecyne. Application of the malonic ester synthesis to the corresponding 1,14-diiodo compound furnishes 9-octadecynedioic acid, which on semi-hydrogenation gives *cis*-9-octadecenedioic acid. Hydroxylation with performic acid forms phloionic acid; hydroxylation with permanganate forms the stereoisomer. The over-all yield of phloionic acid from 8-chloro-1-octyne is 25–29%.

Zetzsche and his co-workers[2,3] showed by degradation that phloionic acid, isolable from cork, has the structure of 9,10-dihydroxyoctadecanedioic acid (VII). The structure was confirmed when phloionic acid as well as its stereoisomer was synthesized by Ruzicka, *et al.*,[4] starting with 9-undecynoic acid, and again by Hunsdiecker,[5] starting with aleuritic acid (9,10,16-trihydroxypalmitic acid). In the present paper we described a new synthesis—starting with 8-chloro-1-octyne—which we have utilized to advantage for the preparation of phloionic acid (*cf.* formulations I–VII).

The first objective was the synthesis of 1,14-dichloro-7-tetradecyne (III) by the coupling of the sodium derivative of 8-chloro-1-octyne (I)[6,7] with 6-iodo-1-chlorohexane (II).

(1) Abstracted from the dissertation submitted by Herbert N. Schlein in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Boston University Graduate School.

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(3) F. Zetzsche and K. Weber, *J. prakt. Chem.*, **150**, 140 (1938).

(4) L. Ruzicka, Pl. A. Plattner and W. Widmer, *Helv. Chim. Acta*, **25**, 1086 (1942).

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