Assignment of the Configuration of Optical Isomers by Gas Chromatography by the Use of Asymmetric Stationary Phases. Absolute Configuration of 2-Amino-3-methylbutane and 2-Amino-4-methylpentane

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The relationship, found previously, correlating the configuration and order of emergence of enantiomeric 2-*N*-trifluoroacetylaminoalkanes on carbonylbis-(*N*-L-valine isopropyl ester) has been confirmed for 2-amino-3-methylbutane (I) and 2-amino-4-methylpentane (II) by unambiguous synthesis of the L(*R*)-isomers. Confusion of assignment due to the effect of polar solvents on the sign of rotation of aminoalkanes has been cleared up for compounds (I) and (II). The findings of Brewster and his co-workers which correlate the sign of rotation [(−)–L(*R*)] have been shown to be correct, provided data referring to the neat liquid are considered, as specified by the authors. Cyclization of 2-aminoalkan-1-ols to aziridines, followed by hydrogenolysis, has been shown to lead to amines of known configuration (*R*:*S* 80:20) from the corresponding α-amino-acids.

THE relation of the sign of rotation (for the neat liquid) with the configuration of 2-amino-n-alkanes was established by Brewster and his co-workers [1] by correlation with the corresponding halides of known configuration through conversions of well defined stereochemical course. The experiments established that the enantiomers of negative rotation have the L†(*R*)-form. On the other hand, Karrer and Dinkel [2] correlated the 2-amino-3-methylbutane (I) and 2-amino-4-methylpentane (II) of positive rotation with L-valine and L-leucine, respectively, leading to the opposite assignment for these two amines. Since, in principle, branching of the chain could lead to this result, this conclusion was not contested at the time.

Subsequently, however, when examining enzymatic reactions and physiological properties of derivatives of (I) and (II), the assignment of Karrer and Dinkel led to inconsistencies. Thus, Halpern and his co-workers [3] reported that the L-leucylamides of D-(I) and D-(II) were preferentially hydrolysed by leucine aminopeptidase. These authors later [4,5] reinterpreted this somewhat surprising result by adopting the opposite assignment for (I) and (II), based on an extrapolation of the work of Brewster *et al.*

In a study of the structure–taste relationship of substituted L-isoasparagines [$HO_2CCH_2CH(NH_2)CONHR$], Mazur [6] found an inversion of the configurational requirements for the derivatives of (II), for which, unexpectedly the D-isomer seemed to form a sweet compound. He, thereupon, reinvestigated [7] the assignment of (II), and found a change of the sign of rotation in methanol solution, as compared with the neat liquid. This observation reconciles the apparent contradiction between the results of Karrer and Dinkel, who measured the optical rotation of (II) in methanol [(+)-L(*R*)], and Halpern *et al.*, who reported values for the neat liquid.‡ The correct configuration of (I), however, still remained in doubt.

In our recent publication [9] the relationship between the order of emergence of amino-acid and 2-aminoalkane derivatives on optically active carbonylbis-(*N*-L-valine isopropyl ester) was discussed. On the basis of the data found, the following rule was formulated: the enantiomer emerging first has the substituents at the asymmetric carbon arranged anticlockwise, in descending order of size, when the molecule is viewed in the direction from the carbon to the nitrogen atom. For the case of the 2-aminoalkanes this means that the L(*R*)-enantiomer will have the shorter retention time.

This paper demonstrates the validity of this rule for the branched amines (I) and (II), and concurrently establishes the configuration of (I) as well as providing further supporting evidence for that of (II).

RESULTS AND DISCUSSION

Synthesis of the L-*Amines* (I) *and* (II).—To provide definite confirmation for the proposed g.l.c. assignment, we prepared samples of optically active (I) and (II) from L-valine and L-leucine, respectively, by a sequence of reactions proceeding with retention of configuration. The process (Scheme 1) differs from that of Karrer and Dinkel, by the cyclization step leading from the amino-alcohols to the aziridines and by the ready formation of the free amines by hydrogenation of the latter. The repetition of the correlation of (I) and (II) with the corresponding α-amino-acids was considered mandatory, because there is some uncertainty as to the conditions under which the Swiss authors determined the sign of rotation. Thus, the amine L-(I) is referred to as having a positive $[\alpha]_D$, whereas the only experimental measure-

† In the LD designation of 2-aminoalkanes the assumption is that the 1-methyl group represents the carboxylic function of the corresponding amino-acid, and the longer alkyl radical the amino-acid side chain.

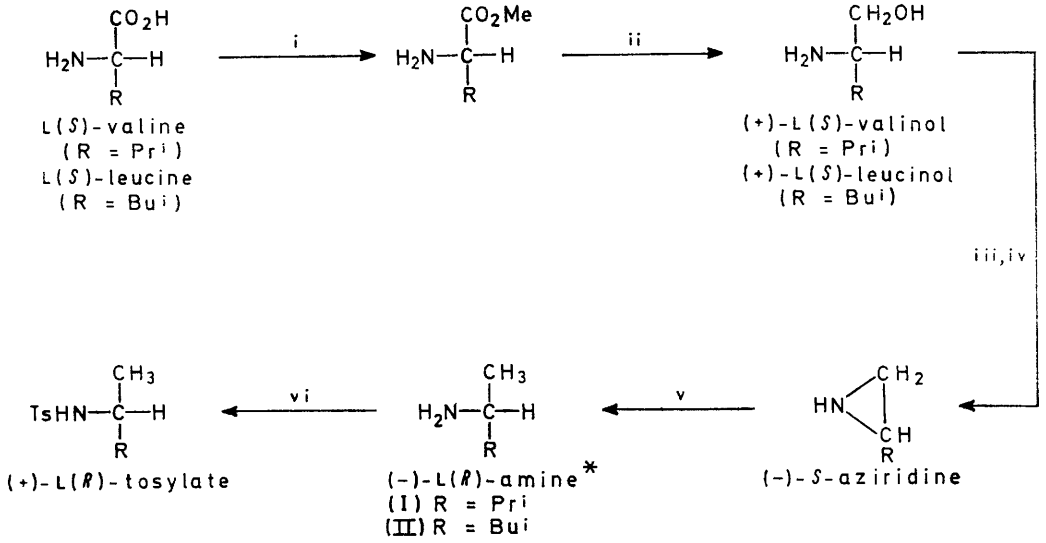
‡ The occasionally deceptive effect of solvents on the rotation of amines is further demonstrated by the observation [8] that the sign of $[\alpha]_D$ of the hydrochloride of 2-amino-octane changes with concentration in non-ionizing solvents.

[1] P. Brewster, F. Hiron, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, *Nature*, 1950, **166**, 179.
[2] P. Karrer and P. Dinkel, *Helv. Chim. Acta*, 1953, **36**, 122.
[3] B. Halpern, J. Ricks, and J. W. Westley, *Chem. Comm.*, 1966, 679.
[4] B. Halpern, personal communication.
[5] J. W. Westley and B. Halpern, 'Gas Chromatography 1968,' ed. S. L. A. Harbourn, Institute of Petroleum, London, 1969, p. 119.
[6] R. H. Mazur, A. H. Goldkamp, P. A. James, and J. M. Schlatter, *J. Medicin. Chem.*, 1970, 1217.
[7] R. H. Mazur, *J. Org. Chem.*, 1970, **35**, 2050.
[8] F. G. Mann and J. W. G. Porter, *J. Chem. Soc.*, 1944, 456.
[9] B. Feibush, T. Tamari, and E. Gil-Av, *J.C.S. Perkin II*, 1972, 1197.

ment mentioned in ref. 2 has a negative value (for the neat liquid). In addition, Scheme 1 appeared to have general interest for the synthesis of amines of known configuration from readily available, optically active α-amino-acids.

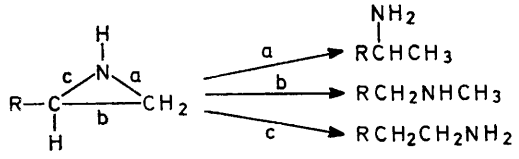
Although the literature is not too explicit on the course of hydrogenolysis of aziridines,[10] it was expected for the rotation of (II) in methanol, as compared with the neat liquid. In contrast, however, it was found, that (I) showed a negative value of rotation both for the neat liquid and the methanol solution.

G.l.c. Assignment.—Optically enriched (I) and (II) were converted to the *N*-trifluoroacetyl derivatives and chromatographed on a capillary column coated with



SCHEME 1 Reagents: i, $SOCl_2$–MeOH; ii, $LiAlH_4$; iii, H_2SO_4; iv, NaOH–KOH; v, H_2–Raney Ni; vi, *p*-$CH_3C_6H_4SO_2Cl$–C_5H_5N.
* Rotation determined for neat liquid.

that this reaction would give some of the desired secondary amines as illustrated in Scheme 2. The



SCHEME 2

hydrogenolysis was conveniently carried out in dioxan, leading mainly to the desired 2-amines (I) and (II) (pathway a), as the principal products. A slight amount of the 1-amines (pathway c) was also formed, whereas the *N*-methylamines (pathway b) were not observed. The degree of retention of configuration was checked by g.l.c. analysis of suitable derivatives of the purified products, as described in the Experimental section. Leucinol did not show more than 1% racemization (Figure 1). On the other hand the free amines were racemized to *ca.* 40—46% (see Figure 2). The relative contribution to racemization of the aziridine formation and the hydrogenolysis could not be established.

All experimental data on rotation given by Karrer and Dinkel for the amino-alcohols, the free amines, and their tosylates had the same sign as found by us. We also confirmed the change of sign, reported by Mazur,[7] carbonylbis-(*N*-L-valine isopropyl ester). As Figure 2 shows, the larger peak which corresponds to the L-isomer, emerges first in both cases. Thus, the chromatographic behaviour is in accord with the prediction of the rule formulated above.

These experiments demonstrate the value of the method and the ease with which it can be applied. An optically enriched mixture obtained, for instance, through partial resolution, is of course required. It should be mentioned that for the amines studied

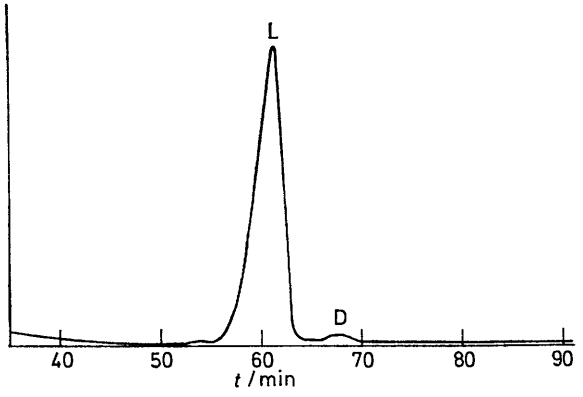


FIGURE 1 Enantiomeric composition of L-leucinol obtained by reduction of L-leucine. Chromatogram of the *O*-pivaloyl-*N*-trifluoroacetyl derivative of leucinol on a 2 m × 4 mm column containing *N*-lauroyl-L-valyl-t-butylamide coated on Chromosorb P, AW-DMCS

[10] P. E. Fanta, in ' Heterocyclic Compounds with Three- and Four-membered Rings,' ed. A. Weissberger, Interscience, New York, 1964, p. 560.

recognition of the relative size of the substituents at the asymmetric carbon atom does not raise any difficulties.*

Empirical correlations of configurations with the relative retention times of diastereoisomers have been found to give consistent results in many cases,[5,11] though occasionally exceptions have been reported.[12,13] Halpern and Westley [5,14] have resolved L-prolylamides of 2-aminoalkanes by g.l.c., and have attempted to correlate the order of emergence with the configuration of the amines. No satisfactory concept was, however, available for linking chromatographic behaviour of these diastereoisomeric amides with their configuration. Thus, these authors could not recognize that their first correlation,[14] based on the assignment of Karrer and Dinkel for (I) and (II) was wrong, nor that their subsequent, opposite one ($r_{LL} < r_{LD}$),[5] based on the work of

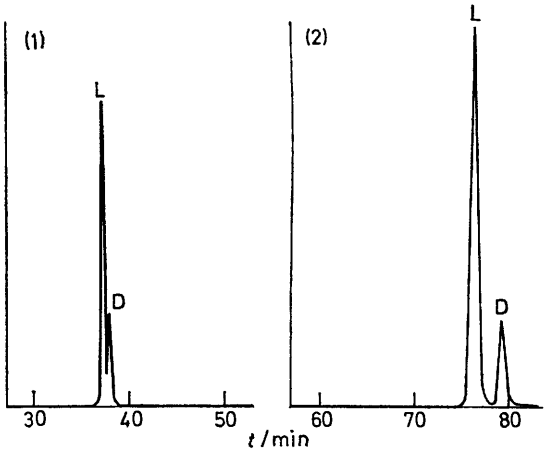


FIGURE 2 Assignment of configuration by the order of emergence on carbonylbis-(*N*-L-valine isopropyl ester). Chromatogram of the *N*-trifluoroacetyl derivatives of the purified amines synthesized from aziridines (Scheme 1). The samples, curve (1) for (I) and curve (2) for (II), were injected separately onto a stainless steel capillary column

Brewster *et al.*, was indeed correct. On the other hand, systematic studies [9] of the behaviour of enantiomers on the chiral phase carbonylbis-(*N*-L-valine isopropyl ester) have permitted the formulation of the rule, cited in the introduction, which, within certain limits, allows the assignment of the configuration of a solute independently of other evidence.

Finally, it should be pointed out again that Brewster *et al.* made their correlation with reference to the signs of rotation measured for the neat liquid. When attention is paid to this provision, it is seen that the assignment for the two amines studied fits this correlation.†

* See ref. 9 for the assessment of the relative size of the substituents in doubtful cases by the use of g.l.c. data.

† The reason for the assignment made by Karrer and Dinkel for (I) is not well understood. A positive sign of rotation, associated with the L-form, is consistent for both (I) and (II), if the $[\alpha]_D$ is measured for the hydrochloride in aqueous solution. It should be mentioned that at one point of ref. 2 the Swiss authors refer, in fact, to L-(I), as having the opposite (+)-rotation as compared with the hydrochloride of D-(I), synthesized by Barrow and Ferguson.[15]

EXPERIMENTAL

M.p.s were determined with a Gallenkamp apparatus and are uncorrected. I.r. spectra were obtained on Perkin-Elmer model 237B or Infracord spectrophotometers. Optical rotations were determined on a Perkin-Elmer model 141 polarimeter. Mass spectra (70 eV) were obtained on an Atlas CH-4 instrument with a direct inlet system or a Finnigan 1015 S/L spectrometer coupled directly to a Varian 1400 g.l.c. system.

Materials.—α-Amino-acid methyl esters. The optically active α-amino-acid (1 mol) was added portionwise with vigorous stirring to a solution of freshly distilled thionyl chloride (120 ml) in methanol (750 ml) prepared and maintained at −40 °C or below. The mixture was allowed to warm to room temperature overnight, then concentrated *in vacuo*. The residue was suspended in dry ether, treated with dry hydrogen chloride, and then filtered, washed with ether, resuspended in ether (1 l), and treated with dry ammonia until saturated. The resulting mixture was filtered, concentrated *in vacuo*, and used in the subsequent reduction step.

Amino-alcohols. (*a*) L-Leucinol. The methyl ester of L-leucine was added dropwise at 0 °C to a suspension of lithium aluminum hydride (35 g) in dry ether (1 l). The resulting mixture was stirred at room temperature overnight and then cautiously treated with water (40 ml) at 0 °C, followed by 15% sodium hydroxide solution (40 ml), and finally with water (120 ml). Stirring at room temperature was continued until a totally white solid resulted, the mixture was then filtered, and the filtrate concentrated *in vacuo*, dried ($MgSO_4$), and distilled. This gave a liquid (51 g), b.p. 109—110·5 °C at 20 mmHg, n_D^{22} 1·4501, $[\alpha]_D$ +3·23 (*c* 10 in EtOH).

(*b*) L-Valinol. The above procedure, carried out on L-valine (1 mol) gave L-valinol (22 g), b.p. 96 °C at 22 mmHg, n_D^{20} 1·4550, $[\alpha]_D$ +16·42° (*c* 10 in EtOH).

Aziridines. (*a*) 2-Isobutylaziridine. A solution of L-leucinol (46·5 g) in water (50 ml) was cooled to 0 °C and sulphuric acid (42 g) in water (50 ml) was added. The resulting solution was left overnight, carefully concentrated *in vacuo*, heated to 150 °C, and maintained at this temperature for 0·5 h. The resulting gum was treated with sodium hydroxide (50 g) in water (70 ml) and steam-distilled. The distillate was saturated with potassium hydroxide and the upper organic layer, which separated, was distilled from potassium hydroxide giving a clear liquid (26 g), b.p. 134—135 °C; n_D^{15} 1·4308°; $\nu_{max.}$ (neat) 3130, 2860, 1440, 1370, and 1350 cm^{-1}, δ 3·1—2·8 (1H, m, NH) and 1·8—0·8 (12H, m, CH), *m/e* 99 (M^+), 98 ($M^+ - H$), 84 ($M^+ - NH$), 70 ($M^+ - NHCH_3$), 56 (major peak, $C_4H_8^+$), and 43 ($C_3H_7^+$), $[\alpha]_D$ −15·6° (*c* 10 in EtOH).

(*b*) 2-Isopropylaziridine. The same procedure gave a clear liquid, b.p. 103—105 °C, $[\alpha]_D$ −26·0° (*c* 10 in EtOH).

Hydrogenolysis of aziridines. (*a*) L-(−)-2-Amino-4-methylpentane (II). Freshly prepared Raney nickel (10 g, wet) was washed with dioxan, then suspended in dioxan (50 ml) and the aziridine (24 g) was added. The resulting mixture was hydrogenated at 70—80 °C and 60 lb in^{-2} for 1·5 h. After filtering off the catalyst, the filtrate was distilled on a spinning band column. Examination of the

[11] E. Gil-Av and D. Nurok, *Proc. Chem. Soc.*, 1962, 146.
[12] R. Charles-Sigler and E. Gil-Av, *Tetrahedron Letters*, 1966, 4231.
[13] B. Feibush and L. Spialter, *J. Chem. Soc.* (*B*), 1971, 106.
[14] B. Halpern and J. W. Westley, *Chem. Comm.*, 1966, **34**.
[15] F. Barrow and G. W. Ferguson, *J. Chem. Soc.*, 1935, 410.

various cuts obtained indicated that separation was incomplete, and hence the amine-rich fractions were further treated by g.l.c. at 110 °C, using a 4 m × 4 mm column containing Chromosorb W coated with 10% SE-30, and mounted in a Hewlett–Packard 7620A Autoprep. The resulting amine (40% racemized) had the following rotations (room temperature): $[\alpha]_D$ −4·77° (neat), −5·89° (*c* in dioxan), −6·61° (*c* 10 in benzene), +4·09° (*c* in 6N-HCl), +0·604° (*c* in MeOH), and +0·625° (*c* 9 in EtOH). The resolution factor of the *N*-trifluoroacetyl derivatives of the enantiomers of (II) on the asymmetric phase at 120 °C is 1·048 and not 1·035, as given erroneously in ref. 16.

(*b*) L-(−)-2-Amino-3-methylbutane (I). The same procedure was followed as above, using the corresponding aziridine (11·0 g) and dioxan (15 ml) with Raney nickel (5 g). The product was distilled and then further separated by g.l.c. as above. The resulting amine (46% racemized) showed the following rotations (room temperature): $[\alpha]_D$ −1·80° (neat), −2·02° (*c* 10 in benzene), −2·48° (*c* 10 in EtOH), and +1·79° (*c* 10 in 6N-HCl).

N-Tosylates. (*a*) L-2-Methyl-4-tosylaminopentane. The purified amine was treated with toluene-*p*-sulphonyl chloride as described.[2] The resulting tosylate was recrystallized from light petroleum giving a solid with m.p. 56·5° (lit.,[2] 62—64°), $[\alpha]_D$ +2·0° (*c* 10 in MeOH), corrected for 40% racemization $[\alpha]_D$ +3·33° (lit.,[2] $[\alpha]_D$ +2·1°) (Found: C, 61·05; H, 8·1; N, 5·45; S, 12·7. Calc. for $C_{13}H_{21}NO_2S$: C, 61·15; H, 8·3; N, 5·5; S, 12·55%).

(*b*) L-2-Methyl-3-tosylaminobutane. The tosylate which was prepared as above was recrystallized from light petroleum (b.p. 40—60 °C) giving a product with m.p. 45—47° (lit.,[2] 45—47 °C), $[\alpha]_D$ +8·9° (*c* 10 in EtOH), corrected for 46% racemization $[\alpha]_D$ +16·5° (lit.,[2] $[\alpha]_D$ +16·7°) (Found: C, 59·75; H, 7·85; N, 5·9; S, 13·3. Calc. for $C_{12}H_{19}NO_2S$: C, 59·75; H, 7·9; N, 5·8; S, 13·3%). A second solid, m.p. 63·5—64 °C, and having the same elemental composition, mass spectrum and, within experimental error, similar rotation, was also isolated. Formation of this solid is probably due to complexities of the binary diagram of the L- and D-tosylates.

G.l.c.—Determination of the configuration of the amines by g.l.c. The amines purified by preparative g.l.c. showed ⩽1% impurities (by g.l.c.). A sample (1 g) of amine was added to methylene chloride (10 ml) and cooled by liquid nitrogen. Trifluoroacetic anhydride (2 ml) was added and the resulting mixture allowed to warm to room temperature, left for 2 h, and then concentrated *in vacuo*. The residue was taken up in ether (15 ml), washed successively with water (5 ml), 5% sodium carbonate solution (5 ml), water (5 ml), and dried ($MgSO_4$). Chromatography was carried out using a 450 ft × 0·02 in capillary column coated with a 5% ether solution of the optically active ureide phase at 120° (see Figure 2).

Determination of L-*leucinol racemization.* A solution of L-leucinol (400 mg) in benzene (10 ml) was saturated with dry hydrogen chloride in a thick-walled reaction tube. Then pivaloyl chloride (1 ml) was added and the tube sealed *in vacuo* and heated at 110° overnight. The resulting solution was concentrated *in vacuo*, washed, taken up in methylene chloride (10 ml), and treated as above to form the trifluoroacetyl derivative. Chromatography of this derivative was carried out at 140° on a 2 m × 4 mm stainless steel column containing *N*-lauroyl-L-valyl-t-butylamide [17] coated on 60—80 mesh Chromosorb P, AW-DMCS (1 g of active phase, $[\alpha]_D^{24}$ −21·5°, per 13 g Chromosorb). The retention times of the enantiomers were confirmed by comparison with the derivatives of racemic leucinol. The racemization was 1% (see Figure 1).

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[16] B. Feibush and E. Gil-Av, *J. Gas Chromatography*, 1967, 257.
[17] B. Feibush, *Chem. Comm.*, 1971, 544.