petroleum ether, and then from methanol–H_2O. The yield of pure compounds varies from 3.2 to 5.5 g. (60–80%, based on the carbobenzyloxyamino acid used).

Dipeptides (Compounds 12–22).—The carbobenzyloxy dipepetide benzyl esters are hydrogenated in the usual way,[2] using 80–90% acetic acid as solvent (a volume of 150 ml. per 0.015 mole of compound). Reduction is complete in approximately six hours. The peptides are recrystallized from H_2O (compounds 13, 14, 20), H_2O–ethanol (compounds 14, 15, 17, 18, 19, 21, 22), or 90% methanol and ether (compounds 12, 16). The yield of the individual pure peptides varies from 2.3 to 3.5 g. (70–85%).

Chromatography of Peptides.—Ascending, one dimensional, paper partition chromatography is employed using Whatman No. 1 paper and two solvent systems, (a) phenol–water–NH_3,[18] and (b) butanol–acetic acid–water (50:10:40).[19] A wad of filter paper is attached to the top of the paper cylinder.[20] This makes it possible to develop the chromatograms for 44–96 hours.

All peptides traveled as single spots in both systems. The α- and γ-isomers are readily separable in solvent system (b) as indicated by the R_{Glu} values in Table II.

This work was aided by a contract between the Office of Naval Research, Department of the Navy, and Columbia University (NR 124-260).

(18) R. J. Block, *Anal. Chem.*, **22**, 1327 (1950).

(19) C. S. Hanes, F. J. R. Hird and F. A. Isherwood, *Biochem. J.*, **51**, 25 (1950).

(20) J. K. Miettinen and A. I. Virtanen, *Acta Chem. Scand.*, **3**, 459 (1949).

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Benzyl Esters of Glutamic Acid[1]

BY HOWARD SACHS AND ERWIN BRAND

Benzyl esters of glutamic acid (symbol, H·Glu·OH)[2] are useful intermediates in peptide synthesis. The preparation and properties of the L- and D-isomers of the α- and of the dibenzyl esters are presented in this paper.

Experimental[3]

The starting materials, L- and D-glutamic acid,[4] had specific rotations $[\alpha]^{25}D$ +31.6° (1.0% in 6 N HCl), and $[\alpha]^{28}D$ −31.3° (1.3% in 6 N HCl), respectively. All melting points are corrected.

┌OBz
1. **H·Glu·OBz·HCl (L).**—A suspension of 10 g. (0.068 mole) of L-glutamic acid in 150 ml. of benzyl alcohol is warmed to 55°, agitated with a magnetic stirrer while dry HCl is passed in for one hour, and the temperature permitted to rise. The mixture is transferred to a still, and

(1) From a dissertation to be submitted by Howard Sachs in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University. Erwin Brand deceased July, 1953.

(2) For symbols and abbreviations, see the preceding paper by H. Sachs and E. Brand, THIS JOURNAL, **75**, 4608 (1953). *E.g.*, D-glutamic acid-α-benzyl ester, H·Glu·OBz (D); L-glutamic acid-γ-benzyl ester, H·Glu·OH (L); L-glutamic acid dibenzyl ester hydrochloride, H. ⌐OBz
Glu·OBz·HCl (L); N-carbobenzyloxy-L-glutamic acid α-benzyl ester, ⌐OBz
Z·Glu·OBz (L).

(3) We are indebted for analytical work to T. Zelmenis (total and amino N).

(4) D-Glutamic acid was prepared by the enzymatic resolution of acetyl-DL-glutamic acid according to V. E. Price, J. B. Gilbert and J. P. Greenstein, *J. Biol. Chem.*, **179**, 1169 (1949). It was also obtained from DL-pyrrolidone carboxylic acid by alkaloid resolution (G. Hillmann and A. Elies, *Z. physiol. Chem.*, **283**, 31 (1948)); we are indebted to Dr. R. Dische for this material.

75 ml. of benzene added, which is distilled off with most of the H_2O at a bath temperature of about 40°. The mixture is now left *in vacuo* (approximately 10 mm.) for one hour at a bath temperature of 85°. Then, dry HCl is again passed in for one hour as described above. Unchanged glutamic acid hydrochloride (about 2 g.) is now filtered off, benzene added, and the process described previously is repeated. Dry HCl is passed in for a third time; after removal of about one-half of the benzyl alcohol *in vacuo* (steam-bath), the di-ester hydrochloride is precipitated with ether (5–7 volumes), and recrystallized from methanol–ether. The yield of pure compound is 15 g. (61%, not counting the recovered glutamic acid), m.p. 100–102°, $[\alpha]^{22}D$ +9.4° (1.5% in 0.1 N HCl).

Anal. Calcd. for $C_{19}H_{21}O_4N \cdot HCl$ (363.8): N, 3.9; amino N, 3.9. Found: N, 3.9; amino[5] N, 3.9.

┌OBz
2. **H·Glu·OBz·HCl (D).**—This compound is obtained by the same procedure and in similar yield from D-glutamic acid as the L-isomer; m.p. 100–102°, $[\alpha]^{25}D$ −9.0° (2.0% in 0.1 N HCl).

Anal. Calcd. for $C_{19}H_{21}O_4N \cdot HCl$ (363.8): N, 3.9; amino N, 3.9. Found: N, 3.9; amino[5] N, 4.0.

3. **H·Glu·OBz (L).**—Ten grams (0.027 mole) of compound 1, H·Glu·OBz·HCl (L), is dissolved in 100 ml. of
└OBz
glacial acetic acid. Ten ml. (0.12 mole) of constant boiling HI (sp. gr. 1.7) is added and the solution kept at 50° for 5.5 hours. The reaction mixture is taken down *in vacuo* and the resulting oil repeatedly (at least twice) treated with 50 ml. of benzene, which each time is distilled off *in vacuo*. The dark brown sirup is then taken up in 60 ml. of cold (−10°) 95% ethanol containing 7 ml. (0.029 mole) of tri-*n*-butylamine. Additional tri-*n*-butylamine (3–4 ml.) is added to bring the *p*H (moist *p*H paper) to approximately 7, whereupon the product begins to crystallize out. After storing in the ice-box overnight, the product is filtered off and washed copiously with absolute ethanol and ether to give 5.7 g. of crystalline material. The crystals are dissolved at room temperature in 11 ml. of water containing 0.034 mole of HCl, decolorized with charcoal, and an equal volume of absolute ethanol is added. Upon neutralization with tri-*n*-butylamine, crystallization takes place; the mixture is then cooled (0°) for several hours. The yield of pure compound is 4.3 g. (67%), m.p. 147–148°, $[\alpha]^{25}D$ +12.2° (2.9% in 0.1 N HCl).

Anal. Calcd. for $C_{12}H_{15}O_4N$ (237.2): N, 5.9; amino N, 5.9; carboxyl nitrogen,[6] 0.0. Found: N, 5.9; amino N, 5.9; carboxyl nitrogen,[6] 0.0.

4. **H·Glu·OBz (D).**—This is obtained from compound 2 by the same procedure and yield as the L-isomer; m.p. 147–148°, $[\alpha]^{25}D$ −11.9° (2.0% in 0.1 N HCl).

Anal. Calcd. for $C_{12}H_{15}O_4N$ (237.2): N, 5.9; amino N, 5.9; carboxyl nitrogen,[6] 0.0. Found: N, 5.9; amino N, 6.1; carboxyl nitrogen,[6] 0.0.

5. **Z·Glu·OBz (L).**—5.0 g. (0.021 mole) of H·Glu·OBz (L) (compound 3) is suspended in a cooled (0°), and vigorously stirred solution of 3.45 g. (0.025 mole) of K_2CO_3 in 20 ml. of water. When almost all of the ester has dissolved, 4.25 g. (0.025 mole) of carbobenzyloxy chloride is added in four portions over a period of 30 minutes, maintaining the *p*H at approximately 8 by addition of a 10% K_2CO_3 solution (total of 15–20 ml.); and stirring is continued for an additional 10 minutes. The reaction mixture is extracted twice with 30 ml. of ether, and acidified with 6 N HCl, yielding a heavy oil which solidifies on standing. The product is recrystallized from CCl_4 or ethanol–water; yield of pure compound[7] is 5.5–6.6 g. (70–85%), with m.p. 95–96°, $[\alpha]^{24}D$ −10.4° (1.7% in glacial acetic acid).

(5) The compound requires a reaction time of 10 minutes in the Van Slyke, manometric, amino N procedure.

(6) *Cf.* D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941); reaction time with ninhydrin was for seven minutes at *p*H 2.5.

(7) A mixture of Z·Glu·OBz and Z·Glu·OH was obtained as an oil by
└OBz
M. Bergmann, L. Zervas and L. Salzmann (*Ber.*, **66**, 1288 (1933)), by treating N-carbobenzyloxy-L-glutamic anhydride with benzyl alcohol at 100°. W. J. LeQuesne and G. T. Young (*J. Chem. Soc.*, 1954 (1950)) fractionated the mixture with Na_2CO_3 and obtained a solid, m.p. 78–81°, which they considered to be Z·Glu·OBz (L).

Anal. Calcd. for $C_{20}H_{31}O_6N$ (371.4): N, 3.8; neut. equiv., 371. Found: N, 3.7; neut. equiv.,[8] 374.

This work was aided by a contract between the Office of Naval Research, Department of the Navy, and Columbia University (NR 124-260).

(8) Obtained by titration in alcohol; *cf.* E. Brand, B. F. Erlanger and H. Sachs, THIS JOURNAL, **74**, 1851 (1952).

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Separation Factors for Expressing the Relative Adsorbabilities of Liquids on Adsorbents[1]

BY ROBERT W. SCHIESSLER AND CARLETON N. ROWE[2]

Separation factors are used as criteria for the evaluation of various fractional separation processes. By analogy to relative volatility in fractional distillation, the adsorption separation factor, α, is defined as the ratio of relative adsorbabilities and may be expressed as

$$\alpha = (N_A/N_B)^a/(N_A/N_B)^l \qquad (1)$$

where

N = mole fraction
A and B = components
a = adsorbed phase
l = liquid phase

Experimental determination of the adsorbed phase composition cannot be made since a completely satisfactory method for the physical separation of the adsorbed and liquid phases has not been found. In consequence, only one application of the separation factor concept to the adsorption of binary liquid mixtures has been found in the literature. Mair, Westhaver and Rossini[3] have reported separation factors for a number of low molecular weight hydrocarbons. These were determined by a rather indirect method through the use of adsorption columns. A lengthy mathematical treatment of the fractionation process and an independent determination of the adsorbent capacity through the vapor phase were required to employ the fractionation data in an expression similar to equation 1.[4]

(1) American Petroleum Institute Research Project 42. Advisory Committee: H. Sutherland (Chairman), E. M. Barber, J. R. Bates, L. C. Beard, Jr., G. H. Denison, L. M. Henderson, R. F. Marschner, L. A. Mikeska and J. H. Ramser.

(2) American Petroleum Institute Research Fellow. Abstracted from an M.S. thesis by Carleton N. Rowe, 1953.

(3) B. J. Mair, J. W. Westhaver and F. D. Rossini, *Ind. Eng. Chem.*, **42**, 1279 (1950).

(4) The separation factor may be expressed[3] in terms of volume fractions by converting equation 1 as

$$\alpha = \frac{(N_A/N_B)^a}{(N_A/N_B)^l} = \frac{(n_A/n_B)^a}{(n_A/n_B)^l} = \frac{\left[\dfrac{(dv/M)_A}{(dv/M)_B}\right]^a}{\left[\dfrac{(dv/M)_A}{(dv/M)_B}\right]^l} =$$

$$\frac{(v_A/v_B)^a}{(v_A/v_B)^l} = \frac{(V_A/V_B)^a}{(V_A/V_B)^l}$$

where α, N,A,B, a and l have the meanings stated previously

n = moles
v = volume *as liquid* of a component in either the liquid phase or the adsorbed phase
d = *liquid* density of the pure components
M = molecular weight
V = volume fraction

Separation factors determined by the column method tend to be in error since they generally vary with composition due to non-ideality, and wide composition ranges are covered in the column technique.

The present investigation was undertaken to find a direct and more accurate method for determining adsorption separation factors. Jones and Outridge,[5] and Mair, Westhaver and Rossini[3] have observed that the adsorbent capacity determined by equilibration through the vapor phase·is nearly constant for pure liquids having widely different chemical compositions and properties. This has been confirmed in the present work for activated alumina and silica gel. Table I shows the average adsorbent capacities in cc. adsorbed per gram adsorbent for a number of liquids.

TABLE I
ADSORBENT CAPACITIES

Liquid	Alumina Capacity, cc./g.	Alumina Deviation from av., %	Silica gel Capacity, cc./g.	Silica gel Deviation from av., %
Methylcyclohexane	0.213	−0.5	0.357	−1.4
n-Heptane	.218	+1.9	.356	−1.7
Benzene	.217	+1.4	.365	+0.8
Cyclohexane	...	...	.349	−3.6
5-*n*-Butylnonane	.209	−2.3	...	...
Water	.211	−1.4	.383	+5.8
Av.	.214	1.5	.362	2.6

Defining the adsorbed phase in terms of the adsorbent capacity,[6,7] an expression may be derived for determining the separation factor in a static system. In the derivation, the assumption is made that the volumes are additive. Let

A = component preferentially adsorbed
V_A^i = vol. fctn. of A in original liquid mixture
V_A^l = vol. fctn. of A in liquid phase at equilibrium
V_A^a = vol. fctn. of A in adsorbed phase at equilibrium
X = vol. of original liquid mixture in cc.
Y = vol. of liquid phase at equilibrium in cc.
W = weight of adsorbent
z = capacity of adsorbent, cc./g.
Z = Wz, total capacity of W g. of adsorbent in cc.

The material balance for component A at equilibrium is

$$V_A^i X = V_A^l Y + V_A^a (X - Y) \qquad (2)$$

but, as previously defined

$$Z = (X - Y) \qquad (3)$$

thus

$$V_A^i X = V_A^l (X - Z) + V_A^a Z \qquad (4)$$

Rearranging

$$V_A^a = (V_A^i - V_A^l)X/Z + V_A^l \qquad (5)$$

Since the separation factor may be determined from volume fractions[4] and since $V_B^a = 1 - V_A^a$, equation 1 reduces to

$$\alpha = V_B^l V_A^a / V_A^l (1 - V_A^a) \qquad (6)$$

(5) D. C. Jones and L. Outridge, *J. Chem. Soc.*, 1574 (1930).

(6) The assumption that the adsorbent capacity determined by vapor phase equilibration is analogous to the adsorbed phase when the adsorbent is immersed in liquid is slightly erroneous due to the reduction of the vapor pressure of the liquid trapped in fine capillaries.[6]

(7) Mair, Westhaver and Rossini[3] used the adsorbent capacity determined through the vapor phase in their estimation of separation factors from column data.