state of a given multiplicity may react.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada and University of Victoria Faculty Research Fund for financial support. We also acknowledge the contributions of C. F. C. Wong to our initial studies in this area and thank Dr. A. W. Adamson for use of his lifetime apparatus and Dr. G. B. Porter for helpful discussions.

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Received July 25, 1980

Optical Resolution of the D- and L-Amino Acid Family by Liquid-Solid Chromatography

Sir:

Recently, we have reported the development of a technique with a chiral amide-bonded stationary phase [(*N*-acyl-L-valylamino)propyl silica gel] in liquid chromatography, which by recognition of molecular chiralities permits separation of difficult mixtures of enantiomers.[1-3] This technique depends entirely on hydrogen-bond association, and involves no strong complexations such as host–guest,[4,5] metal–chelate,[6-9] and charge-transfer complexations.[10,11] Our results seem to be of interest since hydrogen bonding is frequently involved in enzymatic chiral recognition in spite of its facile and flexible interaction, and most importantly, its weak association energy. The effect of changing *N*-acyl groups on the stationary phase has been tested to obtain the maximum resolution offered by (*N*-formyl-L-valylamino)propyl (FVA) silica gel.[3] We report in this paper that through effective derivatization of solutes the scope of this method can be extended to almost all derivatives in the amino acid family.

Evaluation of derivatizations for improving the resolution was accomplished by systematic N-acylations and O-alkylations of leucine, which was selected as a typical solute as shown in Table I. The separation factors, i.e., enantioselectivity, depended upon changes involving the *O*-alkyl group while the separation factors were not influenced by *N*-acyl substituents. Increasing the bulkiness of *O*-alkyl moieties afforded larger separation factors, with the most effective value being obtained for the *O-tert*-butyl ester.

Thus, we applied the N-acetylation–O-*tert*-butylation procedure to other enantiomers of the amino acid family illustrated in Table II. Binary solvent systems in chromatography were optimized by using stronger components such as diethyl ether, methylene chloride, and chloroform in place of 2-propanol. The nonpolar diluent was *n*-hexane, and solvent mixtures containing 2-propanol were kept at 40 °C, with all others at 20 °C. The most effective separation factors were obtained for separations 1–11 with diethyl ether, for separations 12 and 13 with methylene chloride, and for separations 14 and 15 with chloroform. In each case, other binary solvent systems, such as 2-propanol–*n*-hexane, were tried and found to have smaller separation factors; however, peak separations were still definite in all cases. Some of the results are illustrated in Figure 1.

Table I. Resolution of D- and L-Leucine Derivatives $(CH_3)_2CHCH_2CH(COOR^1)NHCOR^2$ with a FVA Column[a]

derivative		mobile phase, % (v/v) of 2-PrOH in	capacity factor[b] k'		separation factor,[c]
R^1	R^2	*n*-hexane	D	L	*a*
Me	H	6	3.25		1.00
Me	Me	6	3.35	3.66	1.09
Me	Et	4	2.36	2.63	1.11
Me	*i*-Pr	2	3.00	3.39	1.13
Me	*t*-Bu	0.5	2.65	2.93	1.11
Me	Me	4	5.50	6.05	1.10
Et	Me	3	4.98	5.74	1.15
i-Pr	Me	2.5	4.71	5.65	1.20
t-Bu	Me	4	2.03	2.54	1.25
t-Bu	Me	2	4.84	6.70	1.38

[a] The column was 20 × 0.4 (i.d.) cm stainless-steel tubing and was packed by a high-pressure slurry technique.[3] It contained approximately 2 g of FVA silica gel and possessed a dead volume of 1.70 mL with it attached to the liquid chromatograph. The chromatographic runs were made at a constant flow rate of 1 mL/min for doubled FVA columns. The column temperature was 40 °C. In all runs, about 40 μg of racemic leucine derivatives, dissolved in 4 μL of chloroform, was injected onto the column during flow. The appearance of enantiomers in the column eluate was detected by ultraviolet absorption at 230 nm. [b] k' = (retention time of enantiomer − dead time)/dead time. [c] $a = k'$ of L enantiomer/k' of D enantiomer.

Table II. Resolution of Enantiomers of *N*-Acetyl Amino Acid *tert*-Butyl Esters[a]

separation	amino acid	strong solvent in *n*-hexane (%, v/v)	k' D	k' L	*a*	resolution,[b] R_s
1	Leu	Et_2O (80)	3.12	4.33	1.39	4.21
2	Val	Et_2O (80)	3.41	4.69	1.38	4.17
3	Nle	Et_2O (80)	3.13	4.28	1.37	3.99
4	Nva	Et_2O (80)	3.63	4.89	1.35	3.88
5	Abu	Et_2O (80)	4.12	5.37	1.30	3.39
6	Ala	Et_2O (80)	4.83	5.95	1.23	2.66
7	Ile	Et_2O (80)	3.16	4.32	1.37	4.00
8	*O-t*-BuSer	Et_2O (80)	2.13	2.82	1.32	3.12
9	*O*-AcTyr	Et_2O (80)	7.67	9.33	1.22	2.70
10	*O-t*-BuAsp	Et_2O (80)	2.57	3.10	1.21	2.13
11	*O-t*-BuGlu	Et_2O (80)	3.57	4.33	1.21	2.30
12	*S*-BzlCys	CH_2Cl_2 (30)	1.77	2.31	1.31	2.85
13	*N-t*-BuTrp	CH_2Cl_2 (30)	1.88	2.58	1.37	3.50
14	PheGly	$CHCl_3$ (30)	2.28	3.02	1.32	3.18
15	Phe	$CHCl_3$ (30)	1.96	2.71	1.38	3.64
16	*N*-AcLys	2-PrOH (12)	6.83	7.17	1.05	0.60
17	Gln	2-PrOH (8)	17.63[c]		>1.00	<0.30
18	Pro	2-PrOH (4)	2.77		1.00	

[a] The column temperature was 20 °C for separation 1–15, and detection for separations 12–15 was done at 254 nm with all others at 230 nm. Columns and other operating details are as described in Table I legend. [b] $R_s = {}^1/_4(a-1)N^{1/2}[k/(1+k)]$, where k = (k' of D enantiomer + k' of L enantiomer)/2 and N = 16(retention time of enantiomer/bandwidth of peak)^2. [c] Shoulder was definitely detected.

Systems using 2-propanol–*n*-hexane had separation factors in the range of 1.05–1.21 and exhibited weak but sufficient chiral recognition. These values increased dramatically to a maximum of 1.39 when the stronger solvent component was changed from 2-propanol to diethyl ether, methylene chloride, or chloroform. These results suggest that the hydrogen bonding between stationary surface and solute is the determining factor for the enantioselection in aprotic solvents rather than in protic solvents.

The retention of L isomers was always larger than that of D isomers, showing the hydrogen-bond associations between the

(1) Hara, S.; Dobashi, A. *HRC & CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1979**, *2*, 531.
(2) Hara, S.; Dobashi, A. *J. Liq. Chromatogr.* **1979**, *2*, 883.
(3) Hara, S.; Dobashi, A. *J. Chromatogr.* **1979**, *186*, 543.
(4) Sousa, L. R.; Sogah, G. D. Y.; Hoffmann, D. H.; Cram, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 4569.
(5) Sogah, G. D. Y.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 3035.
(6) Davankov, V. A.; Zolotarev, Yu. A. *J. Chromatogr.* **1978**, *155*, 285.
(7) LePage, J. N.; Lindner, W.; Davies, G.; Seitz, D. E.; Karger, B. L. *Anal. Chem.* **1979**, *51*, 433.
(8) Hare, P. E.; Gil-Av, E. *Science (Washington, D.C.)* **1979**, *204*, 1226.
(9) Gilon, C.; Leshem, R.; Tapuhi, Y.; Grushka, E. *J. Am. Chem. Soc.* **1979**, *101*, 7612.
(10) Mikes, F.; Boshart, G.; Gil-Av, E. *J. Chromatogr.* **1976**, *122*, 205.
(11) Pirkle, W. H.; House, D. W. *J. Org. Chem.* **1979**, *44*, 1957.

0002-7863/80/1502-7122$01.00/0

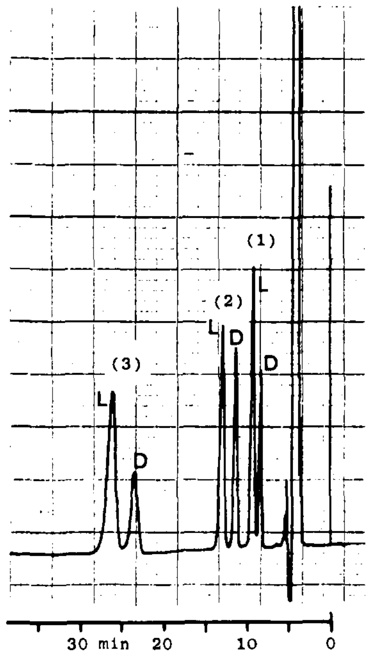

Figure 1. Chromatographic optical resolution of the enantiomeric mixtures of (1) *N*-acetyl-*O*-*tert*-butylserine *tert*-butyl ester, (2) *N*-acetylleucine *tert*-butyl ester, and (3) *N,O*-diacetyltyrosine *tert*-butyl ester. The chromatographic conditions were as described in Table I legend. Mobile phase: 4% (v/v) 2-PrOH in *n*-hexane. A mixture of three pairs of D- and L-amino acid derivatives, each consisting of an enriched concentration of the L enantiomers, was injected onto the FVA column.

stationary surface and L isomers to be more stable. The FVA column was insensitive to the proline derivatives (separation 18) because of the absence of the NH group. Studies on CPK and Büchi's molecular models showed slight conformational differences in diastereomeric associations. In spite of the clear distinction in the association energy of hydrogen bonding and that provided by other stronger and more specific complexations such as host–guest and metal–chelate complexations, the new method has at least as much separation power as previous ones.

The method is based on highly efficient chromatographic technology with nonaqueous phase operation. Through this technology, it has been possible to maximize the number of theoretical plates. Thus, by employing microparticulate packing material and optimizing the solvent systems,[12-14] direct separation of the enantiomers of amino acid derivatives has become feasible. Because of its normal phase operation, prepartive scale separations can be accomplished readily so that the method may be satisfactorily applied to peptide syntheses with high optical purities which is demanded for biologically active substances and drugs.

These results will be useful in the analyses of D-amino acids and D-element-containing peptides which have been found in some microorganisms and are thought to also exist in higher animals.[15] Many families of naturally occurring chiral products containing proton-releasing or proton-accepting groups are of interest on designing chiral stationary phases with minimized recognition powers as well as a maximum number of theoretical plates.

(12) Hara, S. *J. Chromatogr.* **1977**, *137*, 41.
(13) Hara, S.; Fujii, Y.; Hirasawa, M.; Miyamoto, S. *J. Chromatogr.* **1979**, *149*, 143.
(14) Hara, S.; Hirasawa, M.; Miyamoto, S.; Ohsawa, A. *J. Chromatogr.* **1979**, *169*, 117.
(15) Masters, P. M.; Bada, J. L.; Zigler, J. S., Jr. *Nature (London)* **1977**, *269*, 71.

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Received June 30, 1980

Direct Evidence for Ketocarbene–Ketocarbene Interconversion

Sir:

In contrast to the impressive histories of carbonium ion, carbanion, and free-radical rearrangements, the carbene–carbene rearrangement[1] was recorded less than 15 years ago with the report of Shechter's group.[2] Not until 1968 was ketocarbene–ketocarbene rearrangement[3] shown to occur in decomposition of α-diazo ketones, presumably via oxirene by a series of studies of Strausz's group.[4] Interest in this rearrangement has intensified in recent years since the oxirenes, considered[5] a potential 4π antiaromatic system, have been of theoretical and synthetic importance. Nevertheless, only indirect evidence has just been provided for the ketocarbene rearrangement by some sensitive chemical probes, i.e., photochemical decomposition of isotopically labeled α-diazo ketones[4,6] and the thermal and photochemical decompositions of asymmetrically substituted α-diazo ketones,[7] in spite of the fact that the rearranged carbene has been trapped chemically in most of the other systems reported, e.g., aromatic carbene–arylcarbene rearrangement.[1] Moreover, little information exists as to the exact nature of the rearrangement, e.g., the roles of multiplicities and electronic excitation, effects of structure on the relative stability of ketocarbenes in equilibrium, etc. A study is now reported of photochemical processes of three pairs of asymmetrically substituted α-diazo ketones[8] (**1** and **2**) in methanol

1 3 4 2

a, R = Me; **b**, R = Et; c, R = i-Pr

which reveals that (i) the isomeric singlet ketocarbenes (**3** and **4**) from **1** and **2** are actually in equilibrium, (ii) fractional populations are highly dependent upon the substituents on carbenic carbon, and (iii) the rearranged ketocarbene is trapped intermolecularly for the first time.

Direct irradiation of α-diazopropiophenone (**1a**) in degassed methanol through Pyrex filter gave the ester **5** arising from the Wolff rearrangement (WR) product and the OH insertion product **6**. Surprisingly, similar irradiation of positionally isomeric diazo ketone (**2a**) also resulted in the formation of the same reaction

$$\mathbf{1} \text{ or } \mathbf{2} \xrightarrow[\text{MeOH}]{h\nu}$$

$$\underset{\mathbf{5}}{PhCHRCO_2Me} + \underset{\mathbf{6}}{PhCH(OMe)COR} + \underset{\mathbf{7}}{PhCOCH{=}CR_1R_2}$$

a, R = Me, $R_1 = R_2$ = H; **b**, R = Et, R_1 = H, R_2 = Me; **c**, R = *i*-Pr, $R_1 = R_2$ = Me

products (**5** and **6**) in similar yields (Table I). Interestingly, products expected from carbene **4a**, e.g., OH insertion product or vinyl ketone **7a**, were not detected in the reaction mixture. In

(1) See, for review: Jones, W. M. *Acc. Chem. Res.* **1977**, *10*, 353–359.
(2) Vander Stouw, G. G. *Diss. Abstr.* **1965**, *25*, 6974. For a complete report of this work, see: Vander Stouw, G. G.; Kraska, A. R.; Shechter, H. *J. Am. Chem. Soc.* **1972**, *94*, 1655–1661.
(3) See, for review: Meier, H.; Zeller, K. P. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 32–43.
(4) (a) Csizmadia, I. G.; Gont, J.; Strausz, O. P. *J. Am. Chem. Soc.* **1968**, *90*, 7360–7361. (b) Thornton, D. E.; Gosavi, R. G.; Strausz, O. P. *Ibid.* **1970**, *92*, 1768–1769. (c) Frater, G.; Strausz, O. P. *Ibid.* **1970**, *92*, 6654–6656. (d) Fenwick, J.; Frater, G.; Ogi, K.; Strausz, O. P. *Ibid.* **1973**, *95*, 124–132. (e) Csizmadia, I. G.; Gunning, H. E.; Gosavi, R. K.; Strausz, O. P. *Ibid.* **1973**, *95*, 133–137.
(5) Breslow, R. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 565–578.
(6) (a) Zeller, K.-P.; Meier, H.; Kolshorn, H.; Müller, E. *Chem. Ber.* **1972**, *105*, 1875–1886. (b) Zeller, K.-P. *Tetrahedron Lett.* **1977**, 707–708. (c) Zeller, K.-P. *Chem. Ber.* **1979**, *112*, 678–688.
(7) (a) Matlin, S. A.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2623–2630. (b) Cormier, R. A.; Freeman, K. M.; Schnur, D. M. *Tetrahedron Lett.* **1977**, 2231–2234. (c) Timm, U.; Zeller, K.–P.; Meier, H. *Chem. Ber.* **1978**, *111*, 1549–1557.
(8) Diazo ketones **1**[8a] and **2**[8b] were prepared according to the procedure of Regitz and purified by recrystallization just before use. All diazo ketones showed satisfactory spectroscopic data. (a) Regitz, M. *Chem. Ber.* **1965**, *98*, 1210–1225. (b) Regitz, M.; Menz, F. *Ibid.* **1968**, *101*, 2622–2632.

0002-7863/80/1502-7123$01.00/0