found [C. C. Q. Chin and F. Wold, *Arch. Biochem. Biophys.*, **167**, 448–451 (1975)] to undergo quite rapid exchange in deuterium oxide. A significant proportion of enol or enolate anion might therefore be expected to be present in aqueous solutions of the aldehyde **6**.
(27) Thermal racemization and solvolysis of optically active sulfonium compounds have been studied by several investigators: (a) M. P. Balfe, J. Kenyon, and H. Phillips [*J. Chem. Soc.*, 2554–2572 (1930)] concluded that racemization of (−)-ethylmethylphenacylsulfonium halides involved nucleophilic displacement by halide on carbon, followed by recombination to racemic sulfonium salt; (b) D. Darwisch and G. Tourigny [*J. Am. Chem. Soc.*, **88**, 4303–4304 (1966)] studied racemization of *tert*-butylethylmethylsulfonium perchlorate and concluded that loss of chirality proceeded by an internal inversion process since racemization occurs more rapidly than solvolysis; (c) R. Scartazzini and K. Mislow [*Tetrahedron Lett.*, 2719–2722 (1967)] concluded that racemization of 1-adamantylethylmethylsulfonium perchlorate was by pyramidal inversion; (d) D. Darwisch, S. H. Hul, and R. Tomlinson [*J. Am. Chem. Soc.*, **90**, 5631–5632 (1968)] concluded that racemization of several benzylethylmethylsulfonium perchlorates occurred by inversion but that in at least one case, the *p*-methoxybenzyl analogue, racemization was probably by heterolytic bond cleavage via an ion–neutral molecule pair; (e) A. Garbesi, N. Corsi, and A. Fava [*Helv. Chim. Acta*, **53**, 1499–1502 (1970)] provided strong evidence that stereomutation of cyclic sulfonium salts occured by pyramidal inversion at the sulfur atom. We found the *S*-carboxymethylmethionine isomers A and B not readily interconvertible, but a substance having the chromatographic properties of isomer A was produced by heating isomer B in 5 acetic acid at 60 °C; the half-life for interconversion appeared to be somewhat greater than 24 h.
(28) This sample of (*S*)-methionine had $[\alpha]^{25}_{D}$ +23.0° (*c* 1.255, 5 N HCl). S. M. Birnbaum, L. Levintow, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455–470 (1952), reported $[\alpha]^{25}_{D}$ +23.2° (*c* 2.0, 2 N HCl).
(29) W. Barnes and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **29**, 1868–1875 (1973); D. E. Zuccaro and J. D. McCullough, *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **112**, 401–408 (1959); A. T. Christensen and E. Thom, *Acta Crystallogr., Sect. B*, **27**, 581–586 (1971). In this last reference one C–S bond is shortened as a result of delocalization of electrons with the adjacent ring system.
(30) M. C. Caserio, R. E. Pratt, and R. J. Holland, *J. Am. Chem. Soc.*, **88**, 5747–5753 (1966); C. Brown in "Sulfur in Organic and Inorganic Chemistry", Vol. 3, A. Senning, Ed., Marcel Dekker, New York, N.Y., 1972, pp 296–297.
(31) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190–1206 (1958).
(32) The 1.0 M I_2 solutions contained 15 g of KI (0.09 mol) and 12.7 g of I_2 (0.05 mol) in 50 mL solution in distilled water.
(33) H. W. Foote and W. C. Chalker, *Am. Chem. J.*, **39**, 561–567 (1908); H. L. Wells, H. L. Wheeler, and S. L. Penfield, *Z. Anorg. Allg. Chem.*, **1**, 442–455 (1892); G. S. Johnson, *J. Chem. Soc.*, **31**, 249–253 (1877).
(34) P. Coppens in "Crystallographic Computing", F. R. Ahmed, Ed., Munksgaard, Copenhagen, 1970, pp 255–270.
(35) W. C. Hamilton, J. S. Rollett, and R. A. Sparks, *Acta Crystallogr.*, **18**, 129–130 (1965).
(36) "International Tables for X-Ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1962, pp 201–207.
(37) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175–3187 (1965).
(38) D. T. Cromer and D. Liberman, *J. Chem. Phys.*, **53**, 1891 (1970).
(39) H. J. Bernstein, L. C. Andrews, H. M. Berman, F. C. Bernstein, G. H. Campbell, H. L. Carrell, H. B. Chiang, W. C. Hamilton, D. D. Jones, D. Klunk, T. F. Koetzle, E. F. Meyer, C. N. Morimoto, S. S. Sevian, R. K. Stodola, M. M. Strongson, and T. V. Willoughby, "Crysnet—a Network of Intelligent Remote Graphics Terminals", Second Annual AEC Scientific Computer Information Exchange Meetings, Proceedings of the Technical Program, 1974, pp 148–158.
(40) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, The X-RAY System—Version of June 1972, Technical Report TR-192, Computer Science Center, University of Maryland, College Park, Md., 1972; J. M. Stewart, The X-RAY System—Update of 1976.
(41) P. K. Gantzel, R. A. Sparks, R. E. Long, and K. N. Trueblood, UCLALS4 Program in Fortran IV, 1969; H. L. Carrell, ICRFMLS, Modification of UCLALS4, 1975.
(42) H. L. Carrell, VIEW Program in Fortran, 1976.
(43) R. E. Stenkamp and L. H. Jensen, *Acta Crystallogr., Sect. B*, **31**, 857–861 (1975); K. Torii and Y. Iitaka, *ibid.*, **29**, 2799–2807 (1973).
(44) M. Meyers and K. N. Trueblood, *Acta Crystallogr., Sect. B*, **25**, 2588–2598 (1969).

Structural and Isotopic Effects in Hydrophobic Binding Measured by High-Pressure Liquid Chromatography. A Stable and Highly Precise Model for Hydrophobic Interactions in Biomembranes[1]

Nobuo Tanaka and Edward R. Thornton*

Contribution from the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received April 30, 1977*

Abstract: Highly reproducible measurements of partition ratios between aqueous solvents and hydrophobic high-pressure liquid chromatographic columns can be made, thus permitting this system to be used as a model for hydrophobic interactions in biomembranes. The partition ratios have different sensitivities to the addition of a methylene group onto alkyl chains, depending on the mole fraction of methanol added to the aqueous mobile phase. The sensitivity is highest for pure water, but appears simply to be the end of a continuum of hydrophobicity; pure water does not appear to be unique. The free energies of transfer per CH_2 group for these columns resemble those found for binding of alkanes into micelles. There appears to be a cooperative binding effect (fronting rather than tailing of peaks) with alkanes and long-chain carboxylic acids. It is possible to do accurate liquid–liquid chromatography experiments with hydrophobic columns coated with stearic acid, suggesting the possibility of experiments with other substances, including lipids. We have shown isotopic separation of deuterated and protiated palmitic acid and other molecules, and have been able to measure secondary deuterium isotope effects on hydrophobic binding, giving a probe of the nature of the hydrophobic effect.

We report herein our initial studies of hydrophobic effects on a variety of relatively simple molecules, including structural, solvent, and isotope effects, which have given highly reproducible partition constants between mobile and stationary phases.[1a]

The hydrophobic effect is an important structural feature of biological membranes.[2,3] Specific structural factors not found in bulk hydrophobic phases may be involved in membrane phenomena.[2-4] Hydrophobic interactions have been found to be important in determining the activities of a large number of physiologically active substances, and a quantitative linear free energy relationship involving octanol–water partition ratios has been found to have very significant predictive value for structure–activity correlations.[5-8] Chromatographic measures of hydrophobic effects have also been used for structure–activity correlations.[9]

Recently, high-pressure liquid chromatography, using hydrophobic columns consisting of silica particles coated with covalently attached 18-carbon *n*-alkyl chains, has been used to study hydrophobic effects and structure–activity relationships.[10-23]

The nature of the hydrophobic effect has been related to

molecular surface area[24-28] and most recently to molecular volume.[29] ^{13}C NMR relaxation studies have led to the conclusion that water restricts solute molecular motion exceptionally strongly, probably by packing of water molecules tightly around hydrophobic solutes.[30] An interesting correlation of standard free energies of transfer of hydrocarbons from the pure liquid phase to aqueous solution with the number of hydrogens in the hydrocarbon molecule has been found.[31] This correlation suggests a mechanism which closely accounts for the entropy change, involving a requirement that water molecules orient so that one of their unshared electron pairs, and not one of their hydrogen atoms, solvates each C-H bond.[31] It appears that water structuring by the solute is not the major factor in hydrophobicity, however.[32]

Liposomes, closed lipid bilayer vesicles, can be prepared from many kinds of phospholipids and related substances,[3] and these structures serve as important models for biomembrane hydrophobic interactions. Recent studies have measured partition coefficients of nonelectrolytes between liposomes of dimyristoyllecithin and water.[33,34] It was found that lipoproteins solvate alkanes similarly to liposomes and micelles, and differently from liquid hydrocarbon.[35]

We felt that it would be valuable to have a model for biomembrane binding which would permit a wide range of precise studies, including solvent and other effects which could not be systematically explored with liposomes, since liposomes would be unstable if perturbed significantly. A chromatographic method seemed ideal from the point of view of precision and stability, and we therefore decided to use hydrophobic high-pressure liquid chromatography as our model system. Covalent attachment of lipids or related molecules in an appropriate orientation to a solid support appears to be an attractive goal. However, work was first directed toward exploring the properties and reproducibility of the commercially available columns with 18-carbon *n*-alkyl chains covalently attached.

Results

The capacity factor k' is defined by[36]

$$k' \equiv \frac{n_S}{n_M} = \frac{t_R - t_0}{t_0} \quad (1)$$

where n_S and n_M are the amounts of the substance present in the stationary and mobile phases, respectively, and t_R and t_0 are the retention times, at constant flow rate, of the substance and of a completely unretained substance, respectively. The capacity factor is related to the equilibrium partition ratio K as shown in

$$K \equiv \frac{C_S}{C_M} = k' \frac{V_M}{V_S} \quad (2)$$

While k' is directly calculable from the chromatogram, K requires knowledge of V_S, the volume of the stationary phase, which is somewhat ambiguous for interfacial systems such as the hydrophobic columns, though we hope to be able to treat it eventually.

Results obtained for the substances studied are shown in Tables I-V. Columns were either μ-Bondapak C_{18}, packed with 10 μ silica particles, or Bondapak C_{18}/Corasil, packed with 37-50 μ silica particles, to which in either case *n*-octadecyl chains are covalently attached through a silicon-carbon linkage.[37] Chain lengths and other structural features were varied, and mobile phases of water and methanol-water mixtures were investigated.

It was found that results were reproducible to ±1% or better with μ-Bondapak C_{18} and to about ±2% with C_{18}/Corasil. There is some change with time with these columns; however, with care it is only a few percent over a period of several days, and thus it was possible to study large series of related compounds and to demonstrate essentially unchanged k' values for the same substance injected at the beginning and end of the series. Retention times were dependent on sample size, even at relatively low sample sizes, and although the retention time usually (but not always) decreased with increasing sample size, the data did not follow a Langmuir isotherm with any precision. To obtain accurate k' values which could be compared with one another, t_R values were obtained at several sample sizes (typically four to five sizes in ratios up to 20 and up to 1 μmol) and extrapolated by a linear or quadratic least-squares fit to give t_R and then k' values for zero sample size.

Discussion

Much attention was given to reproducibility and variables which might affect the observed retention times. It was found that flow rate had negligible effect on capacity factor in the range, mainly 0.5 mL min^{-1}, where we worked. At small sample size, the chromatogram peaks were symmetrical, but there was tailing at larger sample size for most substances with polar functional groups. For alkanes and some longer chain carboxylic acids, larger sample sizes gave fronting. Tailing is associated with lowered binding at higher concentration, so that the center of the peak moves more rapidly than the wings, and k' decreases with increasing concentration. Conversely, fronting involves increased binding and k' at higher concentration, i.e., binding of some sample onto the stationary phase increases the affinity for binding more sample. Fronting is therefore a cooperativity phenomenon and may bear some resemblance to the binding of hydrophobic molecules together in biomembranes. We hope to explore this phenomenon further.

For accurate k' values, t_R must not be too close to t_0, or $t_R - t_0$ cannot be measured accurately (eq 1). Likewise, if t_R is too long, excessive peak broadening will limit accuracy. A practical range is $0.2 \leq k' \leq 25$. Therefore, different substances were studied in different series of methanol-water mixtures. It can be seen from Tables I and III that there is a significant change in k' with change in solvent. For nonacidic substances, k' was found to be independent of pH, and also independent of ionic strength over the limited range 0-0.01 M studied. For carboxylic acids, studies were generally made at pH ~3, where binding of the acid form was being observed (this was shown by demonstrating that the results were pH independent at values around pH 3). A few studies at higher pH values demonstrated that the anionic form was considerably less tightly bound than the acid form.

Substances containing larger nonpolar portions had higher k' values. It has been shown that free energies of transfer from water to a nonpolar phase for straight-chain substances are linear in the number of carbon atoms in the chain,[3,38] and that log k' (proportional to free energy of transfer) is linear in number of carbon atoms for various homologous series.[11-13] Our data give very precise straight lines at all solvent compositions studied. An example is shown in Figure 1, where it can be seen that chains longer than the 18-carbon chain length of the stationary phase are not exceptional.

This linear free energy relationship can be expressed as

$$\log k' = mn + b \quad (3)$$

where the slope m is the sensitivity to carbon number n and the intercept b is the effect of the groups attached to the ends of the methylene chain. The data for end groups (H, CO_2H), (H, H), and (H, OH) are given in Table II. Several interesting points emerge. The sensitivities increase with more aqueous solvents, suggesting that the hydrophobic effect is a function of the polarity difference between the mobile and stationary phases. The polar head groups have very little effect on the

Table I. Binding of Substances to Hydrophobic μ-Bondapak C_{18} High-Pressure Liquid Chromatographic Column (30 cm), Aqueous Methanol Mobile Phase, 30 °C

Substance	Capacity factor k' (mobile phase[a])		
HCO_2H	0.204 (0; 2.51)		
CH_3CO_2H	0.458 (0; 2.51)		
$CH_3CH_2CO_2H$	0.371 (0.23)	0.463 (0.18)	1.373 (0; 2.51)
$CH_3(CH_2)_2CO_2H$	0.876 (0.23)	1.134 (0.18)	4.092 (0; 2.51)
$CH_3(CH_2)_3CO_2H$	0.620 (0.40)	0.938 (0.33)	2.037 (0.23)
	2.890 (0.18)	13.83 (0; 2.51)	0.652 (0; 6.93)
$CH_3(CH_2)_4CO_2H$	0.545 (0.51)	1.098 (0.40)	1.848 (0.33)
	4.745 (0.23)	7.377 (0.18)	2.319 (0; 6.93)
$CH_3(CH_2)_5CO_2H$	0.840 (0.51)	1.931 (0.40)	3.560 (0.33)
	11.26 (0.23)	19.30 (0.18)	8.283 (0; 6.93)
$CH_3(CH_2)_6CO_2H$	0.535 (0.64)	1.303 (0.51)	3.362 (0.40)
	6.904 (0.33)		
$CH_3(CH_2)_7CO_2H$	0.275 (0.80)	0.742 (0.64)	2.054 (0.51)
	5.907 (0.40)	13.52 (0.33)	
$CH_3(CH_2)_8CO_2H$	0.349 (0.80)	1.020 (0.64)	3.172 (0.51)
	10.23 (0.40)		
$CH_3(CH_2)_{10}CO_2H$	0.556 (0.80)	2.010 (0.64)	7.553 (0.51)
$CD_3(CD_2)_{10}CO_2H$			7.084 (0.51)
$CH_3(CH_2)_{12}CO_2H$	0.902 (0.80)	3.800 (0.64)	
$CH_3(CH_2)_{13}CO_2H$	1.116 (0.80)	5.240 (0.64)	
$CH_3(CH_2)_{14}CO_2H$	1.397 (0.80)	7.289 (0.64)	
$CD_3(CD_2)_{14}CO_2H$		6.770 (0.64)	
$CH_3(CH_2)_{15}CO_2H$	1.770 (0.80)	10.25 (0.64)	
$CH_3(CH_2)_{16}CO_2H$	2.255 (0.80)		
$CH_3(CH_2)_{17}CO_2H$	2.789 (0.80)		
$CH_3(CH_2)_{18}CO_2H$	3.483 (0.80)		
$CH_3(CH_2)_{20}CO_2H$	5.385 (0.80)		
Oleic acid (18:1)	1.529 (0.80)	8.485 (0.64)	
Elaidic acid (18:1 *trans*)	1.597 (0.80)		
Linolenic acid (18:3)	0.898 (0.80)	4.017 (0.64)	
Ricinoleic acid (12h18:1)	0.461 (0.80)	1.852 (0.64)	8.550 (0.51)
9-Hydroxystearic acid (9h18:0)	0.609 (0.80)	2.499 (0.64)	
12-Hydroxystearic acid (12h18:0)	0.593 (0.80)		
$HO_2C(CH_2)_7CO_2H$	0.702 (0.40)		
$HO_2C(CH_2)_8CO_2H$	1.204 (0.40)		
$HO_2C(CH_2)_{12}CO_2H$	0.236 (0.80)	2.722 (0.51)	
$HO_2C(CH_2)_{14}CO_2H$	0.392 (0.80)	1.522 (0.64)	
$CH_3O_2C(CH_2)_{14}CO_2H$	0.654 (0.80)		
$CH_3O_2C(CH_2)_{14}CO_2CH_3$	1.071 (0.80)		
Cyclohexane-CO_2H	1.140 (0.40)	1.887 (0.33)	7.507 (0.18)
1-Adamantane-CO_2H	1.426 (0.51)	3.522 (0.40)	
$C_6H_5CO_2H$	24.68 (0; 2.51)		
$C_6D_5CO_2H$	23.71 (0; 2.51)		
CH_3OH	0.208 (0; 2.51)	0.254 (0; 6.93)	
CH_3CH_2OH	0.507 (0; 2.51)	0.536 (0; 6.93)	
$CH_3(CH_2)_2OH$	0.480 (0.18)	1.359 (0; 2.51)	1.464 (0; 6.93)
$(CH_3)_2CHOH$			1.225 (0; 6.93)
$CH_3(CH_2)_3OH$	1.263 (0.18)	4.246 (0; 2.51)	4.500 (0; 6.93)
$(CH_3)_3COH$			2.692 (0; 6.93)
$(CD_3)_3COH$			2.575 (0; 6.93)
$CH_3(CH_2)_4OH$	3.286 (0.18)	14.16 (0; 2.51)	15.08 (0; 6.93)
$CH_3CH_2C(CH_3)_2OH$			7.522 (0; 6.93)
$CH_3(CH_2)_5OH$	8.551 (0.18)		
C_5H_{12}	0.383 (0.80)	0.928 (0.64)	2.231 (0.51)
	5.360 (0.40)		
C_6H_{14}	0.488 (0.80)	1.286 (0.64)	3.303 (0.51)
	9.682 (0.40)		
C_6D_{14}	3.182 (0.51)	9.249 (0.40)	
C_7H_{16}	0.621 (0.80)	1.770 (0.64)	5.394 (0.51)
	17.72 (0.40)		
C_8H_{18}	0.795 (0.80)	2.466 (0.64)	8.502 (0.51)
C_8D_{18}			8.059 (0.51)
c-C_6H_{12}	2.341 (0.51)	5.543 (0.40)	10.55 (0.33)
c-C_6D_{12}		5.342 (0.40)	10.10 (0.33)
Benzene	0.650 (0.51)	1.211 (0.40)	3.785 (0.23)
	5.144 (0.18)		
Benzene-d_6	3.628 (0.23)	4.907 (0.18)	
$C_6H_5CH_3$	1.000 (0.51)	2.047 (0.40)	3.354 (0.33)
	8.238 (0.23)		
$C_6D_5CD_3$	3.207 (0.33)	7.791 (0.23)	

[a] Mole fraction methanol; followed by pH for pure water. For other compositions, pH (in parentheses) was 0.182 (2.68), 0.229 (2.79), 0.330 (2.96), 0.401 (3.04), 0.510 (3.21), 0.641 (3.33), 0.800 (3.33).

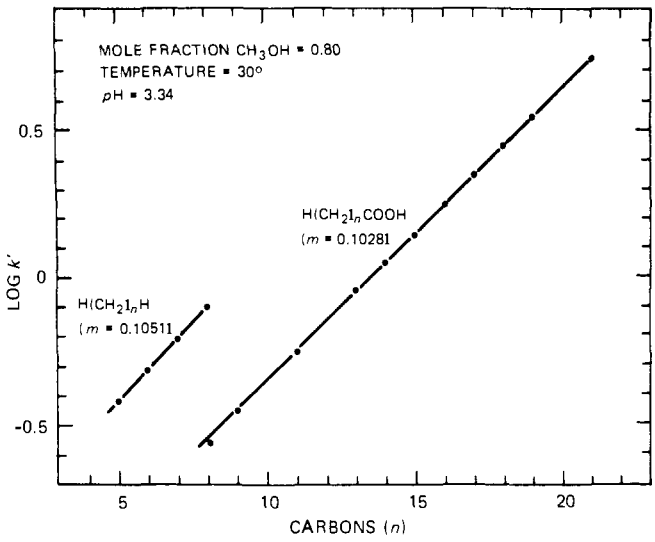


Figure 1. Plots of log k' vs. number of methylene groups for alkanes and carboxylic acids, μ-Bondapak C_{18} column, 30 cm.

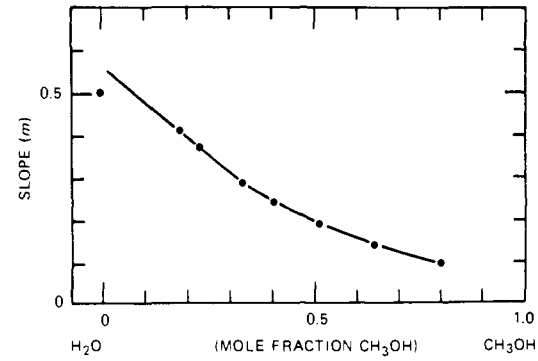


Figure 2. Plot of sensitivity m (eq 3) vs. mole fraction methanol. Data from Table II.

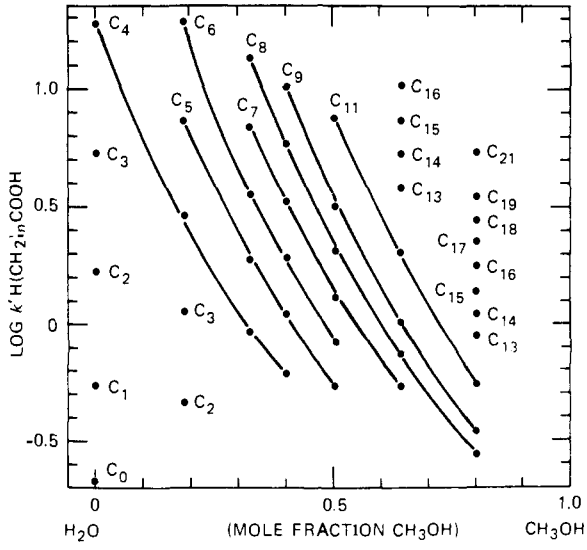


Figure 3. Plots of log k' vs. mole fraction methanol in water, μ-Bondapak C_{18} column, 30 cm.

sensitivities, as expected if the hydrophobic interaction is relatively localized and does not involve long-range hydrogen-bonded cages surrounding the entire molecule. Therefore, the sensitivities are characteristic of the hydrophobic interaction only, and although only limited chain lengths could be studied in pure water, a plot of m vs. mole fraction methanol, Figure 2, indicates that the hydrophobic effect is not really unique to water. There is a continuum of hydrophobic effects in these mixed solvents, and water appears to have a lower effect, if anything (lower m), than the extrapolation from mixtures.

The effects of head groups (b) include possible effects extending to the first methylene group or so, whose environment might be altered by chain-end effects, including specific solvation of polar head groups. This is so since the slopes are effectively derived for insertions into the middle of the methylene chain; it is true that very short chains deviate slightly from linearity with longer chains in a few cases where we have observed a wide enough range of chain lengths. The b values for alkanes (H, H end groups) mainly result from the lower volume of the stationary phase ($V_M/V_S \sim 5$, eq 2), not a hydrophobic contribution from (H, H).

For given end groups, b values tend to change little with solvent composition. The alcohols have more negative b values than acids, and this may reflect dimerization of the acids in the nonpolar, stationary phase. It was possible to study only short chain lengths with accuracy in pure water, but there does appear to be a significantly less negative b for acids and for alcohols in water compared with mixtures. If this interesting effect should prove to be unique to water, it could contribute to a special "hydrophobic" effect associated with water—but it is an unexpected type, since it represents an end-group contribution which is less destabilizing of hydrophobic binding in water than in mixed solvents. It would seem that in some way water solvation is less lost upon binding in the hydrophobic phase, which could be related to the small size or the large capacity for hydrogen bonding of water. However, a number of experimental tests are needed before these present indications can be accepted.

Straight lines intersecting at about 100% methanol have been reported for plots of log k' vs. percent methanol in water, for octanol and hexanol, studied under conditions very similar to ours.[10] Our similar results for carboxylic acids, plotted against mole fraction, are shown in Figure 3. These more extensive data indicate slight curvature, but the curves are nearly parallel. There is some column degradation (small) in our data. Also, our k' values are extrapolated to zero sample size, while the previous work was done at constant sample size;[10] however, the extrapolations to zero sample size alter k' by only a few percent, which is small on the scale of these graphs.

Table II. Sensitivity of Capacity Factor to Chain Length, μ-Bondapak C_{18}, 30 cm × 4 mm, 30 °C, pH 2.5-3.5

Series[a]	Mobile phase (CH_3OH-H_2O) mole fraction CH_3OH	m	b
C_{13}-$C_{19}CO_2H$	0.800	0.099	−1.332
C_8-$C_{13}CO_2H$	0.800	0.103	−1.383
C_5-C_8 alkanes	0.800	0.105	−0.943
C_7-$C_{16}CO_2H$	0.641	0.142	−1.267
C_5-C_8 alkanes	0.641	0.141	−0.737
C_5-$C_{11}CO_2H$	0.510	0.191	−1.219
C_5-C_8 alkanes	0.510	0.194	−0.621
C_4-C_9CO_2H	0.401	0.243	−1.177
C_5-C_7 alkanes	0.401	0.260	−0.569
C_4-C_8CO_2H	0.330	0.289	−1.182
C_2-C_6CO_2H	0.229	0.371	−1.169
C_2-C_6CO_2H	0.182	0.412	−1.191
C_4-C_6OH	0.182	0.416	−1.561
C_2-C_4CO_2H	0.0	0.502	−0.874
C_3-C_5OH	0.0	0.509	−1.398

[a] C_n subscripts refer to number of CH_2 groups in $H(CH_2)_n$-CO_2H, $H(CH_2)_nH$, or $H(CH_2)_nOH$.

The m values can be simply converted to free energy differences per CH_2 group for transfer of methylene from water to the hydrophobic stationary phase, giving −696 and −706

Table III. Binding of Substances to Hydrophobic Corasil C_{18} High-Pressure Liquid Chromatographic Columns (60 cm), Aqueous Buffer Mobile Phase, 30 °C

	Capacity factor k'							
	Untreated column				Silylated column	Stearic acid coated columns[b]		
						0.22%	0.55%	1.00%
	pH[a] 2.53	4.65	6.91	8.08	2.53	2.53	2.53	2.53
Substance	(3.10)	(5.86)	(6.91)[c]	(8.85)	(6.90)	(6.90)	(6.90)	(6.90)
$CH_3(CH_2)_2OH$	1.30	1.25	1.29	1.30	0.64	0.83	0.48	0.44
	(1.24)	(1.30)	(1.12)		(0.61)	(0.83)	(0.49)	(0.44)
$CH_3(CH_2)_3OH$	4.90	5.07	5.08	5.14	2.22	3.02	1.49	1.37
	(5.01)	(4.85)	(4.20)		(2.21)	(2.83)	(1.57)	(1.32)
$CH_3(CH_2)_4OH$	19.66	20.12	20.42	20.81	7.60	10.02	4.89	3.88
	(19.59)	(20.24)	(16.16)	(20.89)	(7.57)	(10.65)	(4.63)	(4.39)
$CH_3(CH_2)_2CO_2H$	2.86	1.84			1.02	1.02	0.50	0.38
	(2.60)	(0.151)						
$CH_3(CH_2)_3CO_2H$	10.78	7.13			3.75	3.71	1.57	1.26
	(10.77)	(0.99)						
$CH_3(CH_2)_4CO_2H$			0.81	0.46	14.36		5.81	4.41
		(4.32)		(0.40)	(0.21)			
$CH_3(CH_2)_5CO_2H$			3.50	1.84				
		(18.93)		(1.80)	(1.24)	(0.49)		
$CH_3(CH_2)_6CO_2H$			14.50	7.08				
				(6.32)	(4.48)	(1.99)	(0.43)	
$CH_3(CH_2)_7CO_2H$								
					(14.96)	(10.18)	(2.08)	(1.55)
$CH_3(CH_2)_8CO_2H$								
							(9.27)	(6.34)
$CH_3CH(OH)CH_2CH_3$	4.24				1.93	2.39	1.28	1.18
$CH_3C(=O)CH_2CH_3$	5.88				3.83	4.07	2.30	2.25
$CH_3CH_2CO_2CH_3$	10.41				6.01	6.30	3.51	3.27
$HO_2C(CH_2)_4CO_2H$	2.51				0.29	0.12		
	(2.35)							
Cyclohexane$(CO_2H)_2$[d]	4.57				0.39	0.20	0.05	
$HO_2C(CH_2)_8CO_2H$			0.40					
$CH_3O_2C(CH_2)_7CO_2H$			27.3					
					(4.20)	(1.56)		
C_6H_5OH					1.43	1.61		
p-$NO_2C_6H_4OH$	11.11	11.70	6.32	1.17	2.79	3.55	1.20	0.96
		(10.62)		(0.44)	(1.40)	(2.10)	(0.54)	

[a] k' values are given in parentheses for the pH in parentheses. [b] % = 100 × wt stearic acid/wt uncoated Corasil C_{18}. [c] 40 °C. [d] Trans-1,4 isomer.

cal/mol for acids and alcohols, respectively. These results can be compared with partition of carboxylic acids between water and heptane (−825 cal/mol), of alcohols between water and the pure liquid alcohol (−821), of alkanes between water and pure liquid alkane (−884), of alkanes between water and sodium dodecyl suflate micelles (−771), and of various amphiphiles between water and their own micellar forms (−732 to −687).[39] The difference in temperature between our data at 30 °C and the latter data at 25 °C is negligible, since the temperature dependence is on the order of 1 cal/deg. Keeping in mind that there are differences between polar head groups, it nevertheless seems that the free energy effects for the chromatographic stationary phase are more like those for micelles than those for bulk liquid phases. These results are also similar to effects for alkane binding to liposomes[34,35] and to lipoproteins,[35] although the series of compounds studied was too limited to warrant quantitative conclusions,[34,35] and in one case the temperature was 0 °C,[35] which may be low enough to have a significant effect on the vesicle properties compared with our experiments at 30 °C.

A few experiments with two columns in series showed that doubling column length had only a few percent effect on k' values, which can be ascribed to slightly different properties for the two columns singly, and had no effect at all on m values.

Also, although the results are not as accurate, the m values for the Corasil C_{18} columns (Table III) were essentially equal to those for μ-Bondapak C_{18} columns (Table I).

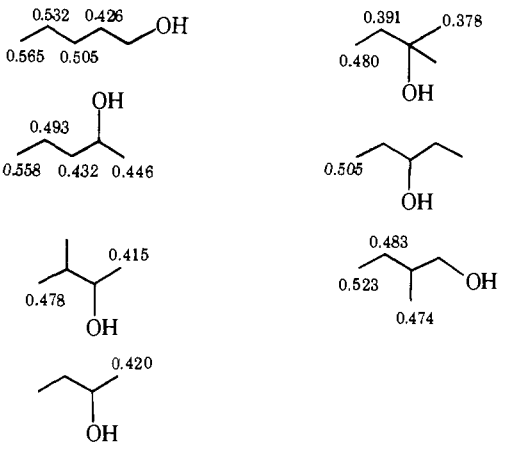
A few temperature effects were measured (Table III), but more work is needed before any quantitative conclusions or comparisons are made regarding hydrophobic interactions at this interfacial layer or conformational properties of the C_{18} chains of the stationary phase. For small solute molecules, partition from water to a hydrophobic phase (liquid hydrocarbon,[3] micelle,[3] or liposome[33,34]) is found to increase with temperature by small amounts but to reverse and decrease with temperature for longer chain solutes.[3] The decreases we have observed for rather short-chain alcohols (Table III) presumably reflect a delicate balance of opposing forces in the chromatographic system, but the main conclusion at present is that enthalpies of transfer are small for all of these systems.

Branched structures provide an additional mode of addition of successive methylene groups to carbon skeletons, and we have studied this effect in various series of alcohols (Scheme I). The data presented are derived under self-consistent conditions, and the $\delta(\log k')$ values are essentially the same as would be calculated from Table I; however, the data were obtained under conditions which were not quite so well controlled as those in Table I, so that a number of the compounds used to obtain the branching effects are not included in Table I. These logarithmic differences, being proportional to free-energy differences, are directly comparable from one structure to another. Both closeness to the polar group and steric crowding can be seen to reduce the hydrophobic effect of a given carbon. Steric crowding will reduce the surface area,[24-28] the effective volume,[29] and probably the accessibility of indi-

Table IV. Binding of Substances to Hydrophobic Corasil C_{18} High-Pressure Liquid Chromatographic Columns (60 cm), Aqueous Methanol Buffer Mobile Phase, Mole Fraction Methanol 0.51, pH 3.21, 30 °C

Substance	Capacity factor k'	
	Silylated column	Untreated column
$CH_3(CH_2)_6CO_2H$	0.52	0.49
$CH_3(CH_2)_7CO_2H$	0.93	0.85
$CH_3(CH_2)_8CO_2H$	1.52	1.42
$CH_3(CH_2)_{10}CO_2H$	4.68	4.17
$HO_2C(CH_2)_{12}CO_2H$	0.80	0.85
C_5H_{12}	1.95	1.64
C_6H_{14}	3.46	2.87
C_7H_{16}	6.02	4.95
C_8H_{18}	10.53	8.50

Scheme I. δ(Log k') for Addition of a Methylene Group in Different Positions[a]



[a] μ-Bondapak C_{18}, 30 cm, H_2O, 30 °C. Methylene group is added to the largest possible precursor in each case.

vidual C–H bonds to water molecules,[31] so the effects of chain branching do not seem to contain features which distinguish among these three mechanisms.

Structural isomers, including cis and trans fatty acids, have significantly different hydrophobic effects (k' values, Table I). However, if one compares structures with four carbons but different numbers and arrangements of oxygens ($CH_3CH_2CO_2CH_3$, $CH_3CH_2COCH_3$, $CH_3CH_2CH_2CH_2OH$, $CH_3CH_2CHOHCH_3$, $CH_3CH_2CH_2CO_2H$, Table III), one finds that the effect of these structural changes is less than the effect of adding a single methylene group to any of them.

We have also studied a number of amino acids and dipeptides; however, data are not included in the tables since the results are incomplete. Significant differences are found among various amino acids, with k' values ordered approximately as might be expected based on side-chain hydrophobic character. Tyrosine and leucine have similar k', much smaller than phenylalanine, which is in turn considerably smaller than tryptophan. The diastereomers of the dipeptide leucylalanine were surpringly different, with k' of 0.13 under essentially the conditions of Table III, pH 5.86, for L,L and D,D, but 1.14 for D,L and L,D.

There are probably some remaining polar sites on the column packings used, which might have some effect on end-group interactions. The polar substances in Table III were indeed found to be significantly less bound by a Corasil C_{18} column which had been trimethylsilylated; however, the m values were changed only a little. The less polar substances

Table V. Isotope Effects for Binding to Hydrophobic μ-Bondapak C_{18} Column (30 cm × 4 mm), 30 °C

Deuterated substance[a]	Mobile phase, mole fraction methanol (pH)	k'_H/k'_D[b]	Isotope effect per D, %[c]
$CD_3(CD_2)_{14}CO_2H$	0.64 (3.33)	1.076	0.24
$CD_3(CD_2)_{10}CO_2H$	0.51 (3.21)	1.066	0.28
C_6D_{14}	0.51 (3.21)	1.036	0.26
C_8D_{18}	0.51 (3.21)	1.054	0.29
C_6D_{14}	0.40 (3.04)	1.049	0.34
c-C_6D_{12}	0.40 (3.04)	1.038	0.31
c-C_6D_{12}	0.33 (2.96)	1.044	0.36
$C_6D_5CD_3$	0.33 (2.96)	1.046	0.56
C_6D_6	0.23 (2.79)	1.043	0.70
$C_6D_5CD_3$	0.23 (2.79)	1.057	0.70
C_6D_6	0.18 (2.68)	1.048	0.79
$C_6D_5CO_2H$	0 (2.51)	1.040	0.78
$(CD_3)_3COH$	0 (6.93)	1.046	0.50

[a] Isotope effects were measured in each case with respect to the corresponding completely protiated substance, in experiments with equal-weight mixtures of protiated and deuterated species, flow rates were generally 0.5 mL min^{-1}. [b] Isotope effects calculated for each sample size and then isotope effects averaged; precision ±0.0005–0.0015. [c] Given by $100[k'_H/k'_D)^{1/n} - 1]$, where n is the number of D atoms substituted for H; precision ±0.02.

studied in aqueous methanol (Table IV) were more tightly bound to the silylated column, and significantly so in the case of alkanes.

Since trimethylsilylation had apparently made the column more hydrophobic, we felt that coating the column with a noncovalently bound hydrophobic substance might make it still more hydrophobic and perhaps give it properties more like lipid bilayer membranes. The covalently bound C_{18} chains would hold the column coating in such a liquid–liquid chromatography experiment very strongly by hydrophobic effects if water were the mobile phase. Initial experiments to test the feasibility and reproducibility of this approach were carried out for convenience with stearic acid (C_{18}), as shown in Table III. With 0.22%, hydrophobicity was increased as shown by higher k' for all substances except carboxylate anions relative to the silylated column. Binding of alcohols was not affected by pH; presumably the stearic acid coating was largely ionized, but the presence of the negative charges did not affect the structure of the stationary phase in a way which influenced hydrophobicity. Carboxylate anions were significantly less strongly bound by the coated column than by the silylated one, suggesting electrostatic repulsion by stearic acid anions. Sensitivities (m) were somewhat smaller for both silylated and stearic acid coated columns than for untreated, but the former were nearly equal. These results demonstrate that coated columns can give excellent stability and reproducibility, suggesting that lipid-coated columns could be studied. In fact, such columns do give very interesting results.[40]

We have previously discussed the isotope effects on hydrophobic binding[1a,41] (Table V) and will not repeat that discussion here. Separations of two substances we studied were recently reported by other workers using similar hydrophobic columns (deuterated benzene and toluene),[42] although baseline resolution was not achieved under their conditions. Recent suggestions of tight packing of water molecules about the solute[30] and of specific solvation of C–H bonds[31] might explain the more restricted motion in the aqueous phase relative to the hydrophobic phase that is indicated by the isotope effects, which relatively favor deuterium over protium in the aqueous phase.[1a] If packing were tighter, or if solvation were more restricting, in the case of unsaturated than of saturated C–H bonds, then the fact that the linear plot of isotope effects per deuterium vs. mole fraction methanol has twice as great a slope

for aromatic compounds as that for aliphatics might also be explained. Interestingly, a distinctive difference in hydrophobic character has been reported for alkanes, which are more hydrophobic than aromatics and other substances which expose electrons to the solvating medium, when compared with molecular volumes.[29] The polarizabilities of the molecular surfaces may be different. It was suggested that solvation by water is different for the two classes, possibly with the oxygens inward in the case of alkanes and the protons inward in the case of aromatics, a so-called reversible-sweater model.[29] To explain the isotope effect differences, the sweater would have to be significantly tighter when the protons point inward.

The fact that isotope effects are higher when the mobile phase is more aqueous demonstrates that they are affected by increasingly restricted motions within the mobile phase as it becomes more aqueous, and thus the effects cannot arise entirely from lipophilic interactions with the stationary phase. However, less restricted motions of the C-H and C-D bonds in the stationary phase would tend to favor protium over deuterium, and this could contribute to the observed isotope effects. Various kinds of C-H bonds do serve as hydrogen-bonding donors,[43] and hydrogen bonding reduces the stretching frequency, which is expected to favor protium over deuterium. Specific solvation of C-H bonds by water[31] would favor protium in the aqueous phase in opposition to the observed isotope effects. Conceivably van der Waals attractions could loosen C-H bonds in the lipophilic phase and contribute to the observed isotope effects, but perhaps the most reasonable source is the relaxation of the highly restricted solute motions characteristic of the aqueous phase[30] upon transfer to the hydrophobic phase. Examination of isotope effects for binding to more highly organized hydrophobic phases, which also more closely resemble biomembrane lipid bilayers, would therefore seem to be important, and we plan to carry out such experiments.

Heavy water, D_2O, has the same electronic structure as H_2O, but the higher mass of deuterium restricts its nuclear motions relative to H_2O, making D_2O more structured. We have carried out a few experiments with D_2O/CH_3CN vs. H_2O/CH_3CN as eluent, and have found that the effects are quite small, but that there is a consistent trend with the D_2O-containing solvent giving a few percent longer retention times. This result is as expected on the basis of hydrophobic effects associated with tight packing of water molecules around the solute,[30] since the more structured D_2O would give still tighter packing.

The present results indicate that more detailed structural knowledge of the hydrophobic column packings would be very desirable.

Experimental Section

Materials. All materials and solvents were commercial materials used without further purification (if they showed no insoluble material and gave only one peak on the chromatogram), or were purified by standard procedures, or in a few cases were synthesized by standard procedures. All solvents were filtered with 0.6 μ polyvinyl chloride filters, except acetonitrile, which was filtered with 0.5 μ polytetrafluoroethylene (bonded to polyethylene net) filters (both from Millipore Co.), before use.

Acid pH was maintained with phosphoric acid in the mobile phase, and higher pH with sodium phosphate buffers as mobile phase, concentrations 0.005-0.01 M.

Columns. Corasil C_{18} was purchased from Waters Associates. Elemental carbon analysis gave 1.5% for this batch, which was noted to give poorer plate counts than obtained in some preliminary experiments using a batch having 0.8% carbon. Silylation was done with Trisil-DMF (Pierce Chemical Co.). Coating with stearic acid was carried out by addition of the Corasil C_{18} to a methanol solution of the acid and evaporation on a rotary evaporator, followed by addition of small amounts of 80% methanol-water to dissolve and evenly distribute apparent crystals of stearic acid, warming, cooling the whole mixture with shaking, and allowing to stand overnight before packing the column. Columns were packed using a tapping method. μ-Bondapak C_{18} columns were purchased from Waters Associates.

Instrument. A Waters Associates ALC 201 liquid chromatograph with septum injector, M6000 pump, and refractive index detector was used. Columns were kept in a constant temperature jacket, and water from a constant temperature bath at 30.05 ± 0.05 or 40.70 ± 0.05 °C was circulated through the jacket. The flow rate accuracy of the pump was verified by volume of timed aliquots.

Measurements. After verification of recorder chart speed accuracy, retention times were measured with a precision steel ruler from the charts. In the case of elution with water (buffered), samples dissolved in pure water were injected to give an unretained (water) peak, caused by the different refractive index of pure water vs. buffer, as a measure of t_0. When elution was with methanol-water, samples dissolved in the same methanol-water solution nevertheless usually gave an unretained peak upon injection because of the small difference in composition between the sample solvent and the eluting solvent.

Values of t_0 are not corrected for the small time lag between column and detector, since the correction is probably of slightly lower precision than the data. True k' values are therefore all 1.1% higher than those tabulated in Table I and all 3.0% higher than those tabulated in Tables III and IV.

Acknowledgments. Support of this research by the National Science Foundation is gratefully acknowledged. We thank Ms. Helena Brachowski for typing the manuscript.

References and Notes

(1) (a) Preliminary communication of part of this work: N. Tanaka and E. R. Thornton, *J. Am. Chem. Soc.*, **98**, 1617 (1976). (b) Supported by the National Science Foundation through Grant MPS72-04818 A03.
(2) S. J. Singer, *Annu. Rev. Biochem.*, **43**, 805 (1974).
(3) C. Tanford, "The Hydrophobic Effect: Formation of Micelles and Biological Membranes", Wiley, New York, N.Y., 1973.
(4) S. N. White, *Nature (London)*, **262**, 421 (1976).
(5) C. Hansch, *Acc. Chem. Res.*, **2**, 232 (1969).
(6) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(7) J. W. Van Valkenburg, *Adv. Chem. Ser.*, **No. 114** (1972).
(8) D. Henry, J. H. Block, J. L. Anderson, and G. R. Carlson, *J. Med. Chem.*, **19**, 619 (1976).
(9) E. Tomlinson, *Chromatogr. Rev.*, **113**, 1 (1975).
(10) B. L. Karger, J. R. Gant, A. Hartkopf, and P. H. Weiner, *J. Chromatogr.*, **128**, 65 (1976).
(11) R. B. Sleight, *J. Chromatogr.*, **83**, 31 (1973).
(12) K. K. Unger, N. Becker, and P. Roumeliotis, *J. Chromatogr.*, **125**, 115 (1976).
(13) E. Tomlinson, H. Poppe, and J. C. Kraak, *J. Pharm. Pharmacol., Suppl.*, **28**, 43P (1976).
(14) H. Hemetsberger, W. Maasfeld, and H. Ricken, *Chromatographia*, **9**, 303 (1976).
(15) C. Horváth, W. Melander, and I. Molnár, *J. Chromatogr.*, **125**, 129 (1976).
(16) W. J. Haggerty, Jr., and E. A. Murrill, *Res./Dev.*, **25**, 30 (1974).
(17) R. M. Carlson, R. E. Carlson, and H. L. Kopperman, *J. Chromatogr.*, **107**, 219 (1975).
(18) J. M. McCall, *J. Med. Chem.*, **18**, 549 (1975).
(19) P. T.-S. Pei, R. S. Henly, and S. Ramachandran, *Lipids*, **10**, 152 (1975).
(20) R. F. Borch, *Anal. Chem.*, **47**, 2437 (1975).
(21) M. J. Cooper and M. W. Anders, *J. Chromatogr. Sci.*, **13**, 407 (1975).
(22) M. S. Mirrlees, S. J. Moulton, C. T. Murphy, and P. J. Taylor, *J. Med. Chem.*, **19**, 615 (1976).
(23) J. N. Done, *J. Chromatogr.*, **125**, 43 (1976).
(24) R. B. Hermann, *J. Phys. Chem.*, **76**, 2754 (1972).
(25) J. A. Reynolds, D. B. Gilbert, and C. Tanford, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 2925 (1974).
(26) D. B. Gilbert, C. Tanford, and J. A. Reynolds, *Biochemistry*, **14**, 444 (1975).
(27) S. H. Yalkowsky and S. C. Valvani, *J. Med. Chem.*, **19**, 727 (1976).
(28) (a) S. C. Valvani, S. H. Yalkowsky, and G. L. Amidon, *J. Phys. Chem.*, **80**, 829 (1976); (b) G. L. Amidon, S. H. Yalkowsky, S. T. Anik, and S. C. Valvani, *ibid.*, **79**, 2239 (1975); (c) G. L. Amidon and S. T. Anik, *J. Pharm. Sci.*, **65**, 801 (1976).
(29) A. Leo, C. Hansch, and P. Y. C. Jow, *J. Med. Chem.*, **19**, 611 (1976).
(30) (a) O. W. Howarth, *J. Chem. Soc., Chem. Commun.*, 286 (1974); (b) O. W. Howarth, *J. Chem. Soc., Faraday Trans. 1*, **71**, 2303 (1975).
(31) S. J. Gill and I. Wadsö, *Proc. Natl. Acad. Sci. U.SA.*, **73**, 2955 (1976).
(32) D. Patterson and M. Barbe, *J. Phys. Chem.*, **80**, 2435 (1976).
(33) Y. Katz and J. M. Diamond, *J. Membr. Biol.*, **17**, 69, 87, 101 (1974).
(34) J. M. Diamond and Y. Katz, *J. Membr. Biol.*, **17**, 121 (1974).
(35) W. L. Stone, *J. Biol. Chem.*, **250**, 4368 (1975).
(36) See B. L. Karger, L. R. Snyder, and C. Horvath, "An Introduction to Separation Science", Wiley, New York, N.Y., 1973, pp 30, 130-131.

(37) Manufactured by Waters Associates, Milford, Mass.
(38) R. Smith and C. Tanford, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 289 (1973).
(39) Reference 3, pp 50–51.
(40) M. F. Czarnieckl and E. R. Thornton, to be published.
(41) An error in the legend of Figure 1 of ref 1a should be noted. The sample size for this baseline separation was smaller. It was in fact 25 μL of a solution ca. 4 mM in each acid, or 1 μmol of each.
(42) G. P. Cartoni and I. Ferretti, *J. Chromatogr.*, **122**, 287 (1976).
(43) F. M. Slasinski, J. M. Tustin, F. J. Sweeney, A. M. Armstrong, Q. A. Ahmed, and J. P. Lorand, *J. Org. Chem.*, **41**, 2693 (1976).

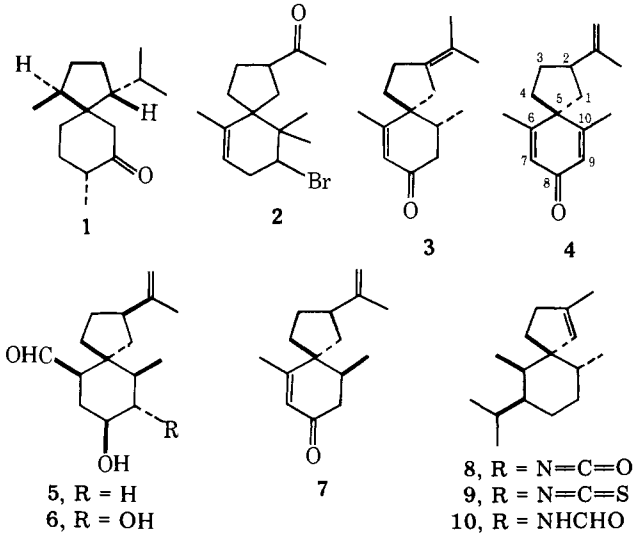
A General Method of Preparing Functionalized Spirocycles. Synthesis of Spirovetivane Sesquiterpenes[1,2]

William G. Dauben* and David J. Hart

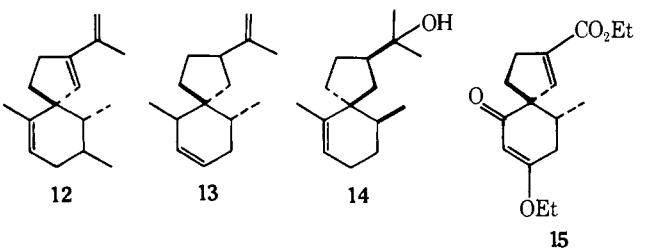
Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received March 17, 1977*

Abstract: The reaction between the sodium salts of α-formylcycloalkanones and 1-carbethoxycyclopropyltriphenylphosphonium tetrafluoroborate has been found to produce moderate yields of spirocycles. The application of this reaction to the total synthesis of the sesquiterpenes (±)-β-vetivone, (±)-hinesol, (±)-β-vetispirene, and (±)-α-vetispirene via a common intermediate is discussed.

A large number of sesquiterpenes possessing a spiro[4.5]-decane carbon skeleton have been characterized during the past 20 years.[3] These natural products can be divided into four classes based upon the location of alkyl substituents on the spiro[4.5]decane nucleus. The acoranes and the enantiomerically related alaskanes constitute the largest class of naturally occurring spir[4.5]decanes (e.g. acorone (**1**)).[3] Spirolaurenone (**2**), a halogenated sesquiterpene recently isolated from the marine plant *Laurencia glandulifera*, is the sole member of a second type of spiro[4.5]decane sesquiterpene.[4] The spirovetivanes, a third class of these sesquiterpenes, have been isolated from a variety of sources such as the essential oil of the Indian grass *Vetiveria zizanioides* (e.g., β-vetivone (**3**)).[3] Recently, anhydro-β-rotunol (**4**),[5] lubimin (**5**),[6a-c] oxylubimin (**6**),[6a,7] and solavetivone (**7**),[5] have been isolated as stress metabolites from potato tubers infected with the blight fungus *Phytophoria infestans*.[8] It has been demonstrated that lubimin possesses antifungal properties and it, as well as the other spirovetivanes produced by these potatoes, may be involved in the defense mechanism of the potato against various pathenogens. Also, an interest has been expressed in assaying these metabolites for their mammalian toxicity.[9] Finally, spiranes **8**–**10**, known as the spiroaxanes, were recently isolated from the marine sponge *Axinella cannabina*.[10]



The spirovetivanes, spiroaxanes, and spirolaurenone are related in the respect that they all have alkyl substituents attached to C-2 of the spiranyl moiety. Many approaches to spiro[4.5]decanes of this type have been evaluated, a number of which culminated in successful syntheses of one or more natural products.[3,11] Owing to the increasing occurrence of spiro[4.5]decanes possessing interesting biological activity, the development of efficient routes to such spirocycles has been actively pursued. In this laboratory the investigation of the reaction between α-formylcycloalkanones and 1-carbethoxycyclopropyltriphenylphosphonium tetrafluoroborate (**11**)[12] has led to the development of a new route to a variety of 2-substituted spiro[4.5]decanes. This reaction has also been used in rapid, stereospecific syntheses of the spirovetivanes α-vetispirene (**12**),[11b,13] β-vetispirene (**13**),[13,14] β-vetivone (**3**),[15] and hinesol (**14**),[16] via the pivotal intermediate **15**.[17]



It had been reported that when enolates of β-keto esters and symmetrical β-diketones were allowed to react with **11**, excellent yields of cyclopent-1-enecarboxylates were obtained.[12] Since it has been established that stabilized phosphoranes exhibit greater reactivity toward aldehydic than toward ketonic carbonyl groups,[18] it was anticipated that α-formylketone enolates might react with **11** to produce spiranyl vinylogous β-keto esters.[19] It was found that when the sodium salt of α-formylcyclohexanone (**16**) and **11** were allowed to react in HMPT, spiro keto ester **17** was produced as the major isolable non-phosphorus-containing compound. This reaction presumably involves nucleophilic attack of the enolate on the geminally activated cyclopropane to produce a stabilized phosphorus ylide which then undergoes a regiospecific intramolecular Wittig reaction at the aldehyde carbonyl group. No products arising from closure at the ketone carbonyl group were detected. In addition to **17**, small amounts of cyclohexanone and 4-carbethoxy-2,3-dihydrofuran (**18**) were ob-