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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF FENTANYL HOMOLOGUES AND ANALOGUES

II. VARIABLES AFFECTING HYDROPHOBIC GROUP CONTRIBUTION*

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SUMMARY

The effects of organic modifier, stationary phase, hydrophobic substitution, and temperature on the group contribution values for 26 homologues and analogues of the drug fentanyl were studied. Using equations relating group contribution to molecular connectivity, it was found that hydrophobic selectivity is approximately independent of mobile phase composition for mixtures commonly employed in solvent optimization schemes based on overlapping resolution mapping. Similarly, hydrophobic selectivity was also found to be almost identical on both a silica-based Partisil 10-ODS-3 column and a polymer-based PRP-1 column under normalized time conditions. In contrast, hydrophobic selectivity was found to depend on the position of methylene substitution on the parent fentanyl molecule and the type of substituent. For all mobile phases studied there is a small decrease in group contribution values with increases in temperature.

INTRODUCTION

In the course of our work it became desirable to study the reversed-phase high-performance liquid chromatographic separation of 26 homologues and analogues of fentanyl, a powerful analgesic. Because these compounds differ primarily in the location of methyl, fluoro, and methylene groups on the benzene, aliphatic, and alicyclic portions of the fentanyl molecule, it was of interest to study their retention changes in reversed-phase chromatographic systems. Reversed-phase systems exhibit a marked selectivity for the hydrocarbon structure of solutes.

Studies relating the effects of mobile phase composition on hydrophobic selectivity have for the most part been limited to binary mixtures[1–3]. Bakalyar *et al.*[4], Colin *et al.*[5] and Glajch *et al.*[6] have examined some ternary mixtures. In this work

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we examined the effect of binary, ternary, and quaternary mobile phases on hydrophobic selectivity. The mobile phases comprised binary solvents, or mixtures of binary solvents at constant solvent strength, and were those normally utilized in solvent optimization schemes based on overlapping resolution mapping[6,7].

There is disagreement as to the effect of a chemically bonded stationary phase on hydrophobic selectivity. Several authors[8–11] believe that selectivity depends on the length or carbon loading of the alkyl bonded phase, while others[12] believe that no selectivity differences are found for solutes of comparable structures. Therefore it was of interest to examine hydrophobic selectivity on both a silica-based column and a polymer-based column.

EXPERIMENTAL

The liquid chromatograph employed in this study consisted of the following components: a Model 8800 4-solvent gradient system with oven (DuPont), a Model LC85 variable-wavelength UV detector at 254 nm containing a 2.5-μl flow cell, a ISS-100 autosampler (Perkin-Elmer) and a Sigma 15 data system interfaced with a Model 3600 data station (Perkin-Elmer).

All experiments were performed on a 25 cm × 4.6 mm I.D. stainless-steel column with 10 μm C_{18} packing material (Partisil 10-ODS-3, Whatman) or a 15 cm × 4.6 mm I.D. stainless-steel column with 10 μm polystyrene–divinylbenzene copolymer (PRP-1, Hamilton).

Acetonitrile, methanol and tetrahydrofuran THF were from Burdick & Jackson "distilled-in-glass". Other chemicals were reagent grade. The various homologues and analogues of fentanyl were synthesized in this laboratory as the hydrochloride salts. The 2-methyl homologue was obtained from Dr. Thomas Riley of the University of Mississippi.

For experiments reported here binary or ternary component mobile phases consisting of a phosphate buffer and organic components were employed. In the instrument solvent 1 consisted of water. Solvent 2 consisted of a concentrated phosphate buffer comprised of 16 parts water, 3 parts 2 *M* sodium hydroxide and 1 part phosphoric acid. Solvents 3 and 4 consisted of organic solvent as specified. Solvent 2 was kept constant at 20% of total volume for all experiments. For the quaternary mobile phases solvent 1 consisted of phosphate buffer (maintained at 20% of total volume) and solvents 2–4 were pure organic components as specified.

All experiments were carried out at 40°C unless otherwise specified.

The individual homologues and analogues were dissolved in a solvent weaker than the mobile phase and were coinjected with fentanyl. The structures of the various compounds studied are presented in Table I.

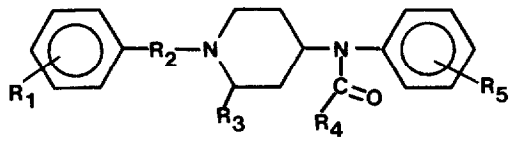
RESULTS AND DISCUSSION

Relationship between group contribution and connectivity

A group contribution approach[2,13,14] has been utilized to study the effect of substituent substitution on retention. The substituents studied include methyl, methylene, and fluoro groups. The group contribution value τ is defined by:

TABLE I

STRUCTURE OF FENTANYL HOMOLOGUES AND ANALOGUES



Compound	R_1	R_2	R_3	R_4	R_5
1	–	CH_2	–	CH_3	–
2	–	CH_2	–	CH_2CH_3	–
3*	–	CH_2CH_2	–	CH_2CH_3	–
4	–	CH_2CH_2	–	CH_3	–
5	–	$CH_2CH_2CH_2$	–	CH_3	–
6	–	$CH_2CH_2CH_2$	–	CH_2CH_3	–
7	–	$CH_2CH(CH_3)$	–	CH_2CH_3	–
8	–	CH_2CH_2	–	CH_3	*o*-CH_3
9	–	CH_2CH_2	–	CH_3	*m*-CH_3
10	–	CH_2CH_2	–	CH_3	*p*-CH_3
11	–	CH_2CH_2	–	CH_2CH_3	*o*-CH_3
12	–	CH_2CH_2	–	CH_2CH_3	*m*-CH_3
13	–	CH_2CH_2	–	CH_2CH_3	*p*-CH_3
14	*o*-CH_3	CH_2CH_2	–	CH_3	–
15	*m*-CH_3	CH_2CH_2	–	CH_3	–
16	*p*-CH_3	CH_2CH_2	–	CH_3	–
17	*o*-CH_3	CH_2CH_2	–	CH_2CH_3	–
18	*m*-CH_3	CH_2CH_2	–	CH_2CH_3	–
19	*p*-CH_3	CH_2CH_2	–	CH_2CH_3	–
20	–	CH_2CH_2	CH_3	CH_2CH_3	–
21	–	$CH(CH_3)CH_2$	–	CH_3	–
22	–	$CH(CH_3)CH_2$	–	CH_2CH_3	–
23	–	CH_2CH_2	–	CH_3	*o*-F
24	–	CH_2CH_2	–	CH_3	*m*-F
25	–	CH_2CH_2	–	CH_2CH_3	*m*-F
26	–	CH_2CH_2	–	CH_2CH_3	*p*-F

* Fentanyl.

$$\tau = \log k'_s - \log k'_{pa} \quad (1)$$

where k'_s is the capacity factor of a solute differing in a substituent group from a parent compound and k'_{pa} is the capacity factor of the parent compound, fentanyl.

For reversed-phase ion-pair chromatography involving hydrophobic selectivity, Riley *et al.*[12] derived the following relationship from Horváth's solvophobic theory[15]:

$$\tau = K_2 \Delta(\Delta HA) \quad (2)$$

where K_2 is a constant related to surface tension of the mobile phase and $\Delta(\Delta HA)$ is the difference in hydrocarbonaceous contact area between solute and stationary phase caused by the absence or presence of one or more substituents. The contact area is shown to be proportional to the surface area of the molecule[16]. A topological

index related to bond orders and electronic valences known as molecular connectivity[17] is utilized to represent molecular surface areas[18]. Wells *et al.*[19] correlated the connectivity indexes of *n*-alkylbenzamides with their contact areas. Karger *et al.*[1], using a simplified version of the connectivity index employed in this study, related hydrophobic group contribution for alkyl and alicyclic alcohols to connectivity.

For a given mobile phase eqn. 2 was expressed as follows:

$$\tau = f(\lambda_s - \lambda_{pa}) \tag{3}$$

where λ_s is the connectivity index of a fentanyl homologue or analogue and λ_{pa} is the connectivity index of fentanyl. Using calculated connectivity values for each of the 26 fentanyl compounds (Table II) and experimentally determined values of Tables III and IV, we tested eqn. 3 by calculating linear regressions for experimentally derived chromatographic data from each of seven mobile phases and with both a Partisil 10-ODS-3 and a PRP-1 column.

We first attempted to correlate τ with connectivity for the chromatographic separation employing a THF–buffer mobile phase with a Partisil 10-ODS-3 column using first order path connectivity indexes. The correlation coefficient and regression line we obtained were:

$$\tau = 0.47\Delta^1\lambda_p + 0.020 \; (n = 26, r = 0.916) \tag{4}$$

where $^1\lambda_p$ represents first order path connectivity.

When the compounds were divided into two classes, one containing compounds with substitution in the aliphatic or alicyclic portion of the molecule and the other substitution on the benzene ring, the following values were obtained:

$$\tau = 0.41\Delta^1\lambda_p - 0.015 \; (n = 10, r = 0.971) \tag{5}$$
(aliphatic or alicyclic substitution)

$$\tau = 0.55\Delta^1\lambda_p + 0.029 \; (n = 17, r = 0.881) \tag{6}$$
(aromatic substitution)

For the aliphatic- and alicyclic-substituted compounds a further improvement in correlation was obtained by adding a third order cluster connectivity term, $^3\lambda_c$, which is inversely proportional to molecular branching:

$$\tau = 0.41\Delta(^1\lambda_p + 1/^3\lambda_c) + 0.028 \quad (n = 10, r = 0.985) \tag{7}$$

For the aromatic-substituted compounds, an improvement in correlation was obtained by using a valence term which is sensitive to fluoro substitution:

$$\tau = 0.52\Delta^1\lambda_p^v + 0.067 \quad (n = 17, r = 0.956) \tag{8}$$

where $^1\lambda_p^v$ represents first order path valence connectivity.

A further improvement in correlation was obtained for the aromatic-substituted compounds by adding a third order cluster-valence term which is sensitive to methyl substitution on a ring:

TABLE II

CALCULATED CONNECTIVITY TERMS FOR FENTANYL HOMOLOGUES AND ANALOGUES

Compound	$^1\lambda_p$	$1/^3\lambda_c$	$^1\lambda_p^v$	$^3\lambda_c^v$
1	9.75	0.80	8.33	0.63
2	10.29	0.92	8.89	0.60
3	10.79	0.92	9.39	0.60
4	10.25	0.80	8.83	0.63
5	10.75	0.80	9.33	0.63
6	11.29	0.92	9.89	0.60
7	11.20	0.77	9.82	0.69
8	10.66	0.68	9.25	0.76
9	10.65	0.65	9.25	0.80
10	10.65	0.65	9.25	0.80
11	11.20	0.77	9.81	0.73
12	11.19	0.72	9.81	0.77
13	11.19	0.72	9.81	0.77
14	10.66	0.68	9.25	0.75
15	10.65	0.65	9.25	0.80
16	10.65	0.65	9.25	0.80
17	11.20	0.77	9.81	0.72
18	11.19	0.72	9.81	0.77
19	11.19	0.72	9.81	0.77
20	11.20	0.77	9.82	0.75
21	10.66	0.68	9.26	0.72
22	11.20	0.77	9.82	0.69
23	10.65	0.68	8.94	0.67
24	10.65	0.65	8.94	0.69
25	11.19	0.72	9.50	0.66
26	11.19	0.72	9.50	0.66

$$\tau = 0.50\Delta(^1\lambda_p^v + {}^3\lambda_c^v) - 0.0002 \quad (n = 17, r = 0.960) \tag{9}$$

For the same mobile phase with a PRP-1 column:

$$\tau = 0.47\Delta(^1\lambda_p + 1/^3\lambda_c) + 0.032 \quad (n = 10, r = 0.985) \tag{10}$$

$$\tau = 0.55\Delta(^1\lambda^v + {}^3\lambda_c^v) - 0.010 \quad (n = 17, r = 0.959) \tag{11}$$

Similar equations were obtained for the six other mobile phases and with both columns as indicated in Table III. Tables IV and V demonstrate that there is generally good agreement between observed and calculated values of τ. Most of the discrepancies were observed on both columns with compounds 4, 8, 11, 13, 17 and 22. Compounds 8 and 11 contain *ortho*-substituted methyl groups which could be forced out of a planar configuration due to steric interactions with the carbonyl alkyl moiety. This could result in a smaller contact area than predicted by connectivity indexes and would account for the fact that calculated values of τ are greater than the observed values.

TABLE III

COEFFICIENTS OF EQUATIONS RELATING GROUP CONTRIBUTION TO CONNECTIVITY INDEXES

Compositions of mobile phases are given in headings of Tables IV and V. Buf = buffer; ACN = acetonitrile; MeOH = methanol, and THF = tetrahydrofuran.

Mobile phase	*Column*	m_1*	c_1*	r_1**	m_2***	c_2***	r_2§	*Equation number*
Buf–ACN	ODS-3	0.33	0.024	0.995	0.35	−0.0030	0.954	12, 13
	PRP-1	0.42	0.025	0.998	0.46	−0.029	0.971	14, 15
Buf–MeOH	ODS-3	0.31	0.025	0.959	0.48	−0.036	0.978	16, 17
	PRP-1	0.41	0.029	0.987	0.54	−0.060	0.978	18, 19
Buf–ACN–THF	ODS-3	0.38	0.027	0.994	0.46	−0.013	0.970	20, 21
	PRP-1	0.48	0.033	0.996	0.55	−0.024	0.973	22, 23
Buf–MeOH–THF	ODS-3	0.37	0.026	0.980	0.49	−0.024	0.962	24, 25
	PRP-1	0.45	0.024	0.998	0.55	−0.036	0.972	26, 27
Buf–ACN–MeOH	ODS-3	0.35	0.028	0.984	0.47	−0.026	0.984	28, 29
	PRP-1	0.47	0.029	0.997	0.55	−0.049	0.979	30, 31
Buf–ACN–MeOH–THF	ODS-3	0.38	0.039	0.986	0.49	−0.017	0.978	32, 33
	PRP-1	0.46	0.032	0.994	0.56	−0.041	0.975	34, 35

* Constant of equation $\tau = m_1 \Delta({}^1\lambda_p + 1/{}^3\lambda_c) + c_1$ ($n = 10$).
** Regression coefficient for equation containing constants m_1 and c_1.
*** Constant of equation $\tau = m_2 \Delta({}^1\lambda_p^v + {}^3\lambda_c^v) + c_2$ ($n = 17$).
§ Regression coefficient for equation containing constants m_2 and c_2.

Effect of mobile phase composition on group contribution

Seven mobile phases were chosen which estimate the coefficients of a cubic equation which describes the relationship between resolution and mobile phase composition. An optimized chromatographic system for the fentanyl homologues and analogues is reported elsewhere[20] in which overlapping resolution mapping is employed with the same solvent systems and a Partisil 10-ODS-3 column. The mobile phase compositions are summarized in the headings of Tables IV and V. The first three mobile phases at equal solvent strength containing a single organic modifier were adjusted to give a k' value for fentanyl of *ca.* 3.5. Mobile phases 4–7 contained equal amounts of binary mobile phases. The organic modifiers consisted of acetonitrile, methanol and tetrahydrofuran.

Although it would be expected that the k' of fentanyl would be 3.5 in mobile phases 4–7, in most instances it was considerably higher. Similar findings for other compounds are reported by Glajch *et al.*[6].

In Table VI the relative amounts of organic modifier required to elute various compounds at constant solvent strength is compared for fentanyl on both columns employed in this study. In addition, Table VI compares the results of our study with other published reports for the solutes benzene and methylthionaphthalene[4,6]. Our results agree with previous findings that solvent strength depends not only on the solvents employed, but on other factors such as solute type and stationary phase[21]. Our calculations are of course based on the assumption that the buffer has the same polarity as water.

For both columns studied, as illustrated in Table III, the similarity of the

TABLE IV

GROUP CONTRIBUTION VALUES FOR PARTISIL 10-ODS-3 COLUMN

Calculated values from eqns. 7, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32 and 33. Abbreviations as in Table III. Obs = observed; calc. = calculated.

Compound	*Buf–MeOH (60:40)*		*Buf–ACN (72:28)*		*Buf–THF (90:10)*		*Buf–MeOH–ACN (66:20:14)*		*Buf–ACN–THF (81:14:5)*		*Buf–MeOH–THF (75:20:5)*		*Buf–MeOH–ACN–THF (75:13:9:3)*	
	Obs.	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*
1	−0.33	−0.33	−0.36	−0.36	−0.45	−0.45	−0.38	−0.38	−0.42	−0.41	−0.40	−0.40	−0.41	−0.41
2	−0.06	−0.13	−0.14	−0.14	−0.14	−0.18	−0.11	−0.15	−0.15	−0.16	−0.11	−0.16	−0.12	−0.16
3	0	0.02	0	0.02	0	0.03	0	0.03	0	0.03	0	0.03	0	0.03
4	−0.25	−0.18	−0.21	−0.17	−0.28	−0.24	−0.26	−0.20	−0.25	−0.22	−0.26	−0.22	−0.26	−0.22
5	−0.06	−0.02	−0.04	−0.03	−0.02	−0.04	−0.05	−0.03	−0.03	−0.03	−0.03	−0.03	−0.04	−0.03
6	0.18	0.18	0.18	0.19	0.26	0.23	0.19	0.20	0.22	0.22	0.23	0.21	0.23	0.22
7	0.06	0.11	0.11	0.11	0.08	0.13	0.09	0.12	0.10	0.13	0.07	0.12	0.09	0.13
8	−0.08	−0.03	−0.07	0	−0.10	0.01	−0.08	−0.02	−0.09	0	−0.09	−0.01	−0.09	−0.01
9	−0.01	0	−0.01	0.02	0	0.03	0	0.01	0	0.02	−0.01	0.01	0	0.02
10	0.02	−0.01	0.01	0.02	0.03	0.03	0.02	0	0.02	0.01	0.03	0	0.03	0.01
11	0.19	0.23	0.16	0.19	0.21	0.28	0.19	0.24	0.19	0.24	0.19	0.25	0.21	0.26
12	0.26	0.25	0.22	0.20	0.30	0.30	0.26	0.25	0.27	0.26	0.28	0.26	0.28	0.27
13	0.29	0.25	0.24	0.20	0.34	0.30	0.29	0.25	0.30	0.26	0.32	0.26	0.32	0.27
14	−0.07	−0.03	−0.04	0	−0.05	0	−0.05	−0.02	−0.04	−0.01	−0.06	−0.02	−0.05	−0.01
15	0.02	−0.01	0.02	0.01	0.04	0.03	0.02	0	0	0.02	0.04	0	0.02	0.01
16	0.02	0.01	0.01	0.01	0.05	0.03	0.03	0	0.04	0.02	0.04	0	0	0.01
17	0.17	0.22	0.17	0.19	0.22	0.27	0.19	0.23	0.20	0.19	0.19	0.24	0.20	0.25
18	0.26	0.25	0.22	0.22	0.31	0.30	0.26	0.25	0.27	0.26	0.28	0.26	0.28	0.27
19	0.27	0.25	0.23	0.22	0.33	0.30	0.27	0.25	0.29	0.26	0.29	0.26	0.29	0.27
20	0.10	0.11	0.11	0.11	0.10	0.13	0.12	0.12	0.12	0.13	0.09	0.12	0.11	0.13
21	−0.06	−0.09	−0.07	−0.10	−0.10	−0.12	−0.07	−0.10	−0.09	−0.11	−0.08	−0.11	−0.08	−0.11
22	0.19	0.11	0.14	0.11	0.19	0.13	0.18	0.12	0.16	0.13	0.18	0.12	0.18	0.13
23	−0.19	−0.22	−0.14	−0.14	−0.17	−0.19	−0.19	−0.20	−0.17	0.19	−0.18	−0.21	−0.19	−0.20
24	−0.22	−0.21	−0.14	−0.13	−0.16	−0.18	−0.20	−0.20	−0.16	0.18	−0.19	−0.20	−0.19	−0.19
25	0.02	0.05	0.09	0.06	0.14	0.09	0.06	0.05	0.11	0.06	0.09	0.06	0.09	0.07
26	0.02	0.05	0.07	0.06	0.16	0.09	0.05	0.05	0.11	0.06	0.09	0.06	0.09	0.07

TABLE V

GROUP CONTRIBUTION VALUES FOR PRP-1 COLUMN

Calculated values from eqns. 10, 11, 14, 15, 18, 19, 22, 23, 26, 27, 30, 31, 34 and 35. Abbreviations as in Table III. Obs. = observed; calc. = calculated.

Compound	*Buf MeOH (51:49)*		*Buf ACN (75:25)*		*Buf–THF (91:9)*		*Buf–MeOH–ACN (64:24:12)*		*Buf–ACN–THF (84:12:4)*		*Buf–MeOH THF (72:24:4)*		*Buf–MeOH–ACN–THF (73:16:8:3)*	
	Obs.	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*	*Obs.*	*Calc.*
1	−0.42	−0.45	−0.46	−0.46	−0.51	−0.51	−0.50	−0.52	−0.52	−0.52	−0.49	−0.50	−0.50	−0.50
2	−0.13	−0.18	−0.19	−0.18	−0.17	−0.20	−0.18	−0.21	−0.19	−0.21	−0.16	−0.20	−0.18	−0.20
3	0	0.03	0	0.02	0	0.03	0	0.03	0	0.03	0	0.02	0	0.03
4	−0.29	−0.24	−0.26	−0.25	−0.32	−0.28	−0.31	−0.28	−0.31	−0.28	−0.32	−0.27	−0.31	−0.27
5	−0.03	−0.04	−0.05	−0.04	−0.01	−0.04	−0.04	−0.05	−0.03	−0.04	−0.03	−0.05	−0.04	−0.04
6	0.26	0.23	0.23	0.24	0.31	0.27	0.27	0.26	0.29	0.27	0.28	0.25	0.28	0.26
7	0.09	0.14	0.15	0.13	0.08	0.15	0.14	0.15	0.13	0.16	0.08	0.14	0.12	0.15
8	−0.13	−0.05	−0.11	−0.02	−0.12	0	−0.13	−0.04	−0.12	−0.01	−0.13	−0.02	−0.12	−0.03
9	−0.05	−0.02	−0.03	0	−0.02	0.03	−0.05	−0.01	−0.02	0.01	−0.03	0	−0.04	0
10	−0.02	−0.03	−0.01	0	0.03	0.02	−0.01	−0.02	0.02	0.01	0	0	−0.04	−0.01
11	0.20	0.24	0.19	0.23	0.22	0.30	0.21	0.26	0.22	0.28	0.21	0.27	0.21	0.27
12	0.26	0.26	0.24	0.24	0.32	0.31	0.27	0.28	0.30	0.30	0.29	0.29	0.30	0.29
13	0.30	0.26	0.27	0.24	0.37	0.31	0.31	0.28	0.34	0.30	0.34	0.29	0.33	0.29
14	−0.10	−0.06	−0.07	−0.02	−0.06	0	0.09	−0.04	−0.07	−0.02	−0.09	−0.03	−0.09	−0.04
15	−0.01	−0.03	−0.01	0	0.02	0.02	−0.01	−0.02	0.01	0.01	0	0	0	−0.01
16	−0.01	−0.03	−0.01	0	−0.02	0.02	−0.01	−0.02	0.01	0.01	0.01	0	0	−0.01
17	0.20	0.23	0.21	0.22	0.25	0.29	0.22	0.23	0.25	0.27	0.23	0.26	0.24	0.26
18	0.28	0.26	0.26	0.24	0.33	0.31	0.29	0.28	0.32	0.30	0.31	0.29	0.31	0.29
19	0.30	0.26	0.27	0.24	0.35	0.31	0.30	0.28	0.33	0.30	0.32	0.29	0.32	0.29
20	0.14	0.14	0.14	0.13	0.12	0.15	0.15	0.15	0.15	0.16	0.12	0.14	0.15	0.15
21	−0.14	−0.12	−0.13	−0.13	−0.11	−0.14	−0.14	−0.14	−0.14	−0.14	−0.14	−0.14	−0.11	−0.14
22	0.17	0.14	0.15	0.13	0.20	0.15	0.17	0.15	0.19	0.16	0.19	0.14	0.18	0.15
23	−0.22	−0.26	−0.17	−0.20	−0.18	−0.22	−0.22	−0.26	−0.20	−0.23	−0.20	−0.24	−0.21	−0.25
24	−0.27	−0.25	−0.18	−0.19	−0.19	−0.21	−0.25	−0.25	−0.21	−0.22	−0.23	−0.23	−0.23	−0.24
25	0.03	0.03	0.12	0.05	0.16	0.08	0.08	0.04	0.13	0.07	0.10	0.06	0.11	0.05
26	0	0.03	0.08	0.05	0.17	0.08	0.06	0.04	0.12	0.07	0.10	0.06	0.09	0.05

coefficients in comparable equations indicates that hydrophobic selectivity within a class of substances is approximately independent of the mobile phases employed in overlapping resolution studies. A similar finding has been reported for binary organic, aqueous solvent mixtures, at the same solvent strength, for alcohols and benzoic acids separated on bonded phase columns[1,2]. Recently it has been reported with bonded phase columns that for various homologous series (*n*-alkanes, *n*-alkylbenzenes, *n*-chloroalkanes, *n*-methylesters and *n*-alcohols) for ternary mixtures derived from two binary mixtures [*e.g.* (1) THF and water and (2) methanol and THF at the same solvent strength] the log α values are more or less constant[5].

Effect of stationary phase on group contribution

Under time-normalized conditions hydrophobic selectivity is nearly independent of stationary phase for the columns employed in this study. As indicated in Table VI, the mobile phases employed with both columns are very similar. Berendsen and De Galan[9] have reported that for a given mobile phase, hydrophobic selectivity will increase with the chain length of a bonded phase column up to about fourteen carbon atoms. Similar findings were reported by Tomlinson *et al.*[10]. This effect has been related to the ability of the bonded phase to interact completely with a solute when its chain length is greater than 14 carbon atoms[9]. A polystyrene–divinylbenzene copolymer, because it is cross-linked and because it might contain long non-polar chains, could interact completely with a solute and could be expected to exhibit selectivity similar in nature to that of a Partisil 10-ODS-3 column.

Effect of hydrophobic substitution on group contribution

The following general trends were observed for the dependence of group contribution values given in Tables IV and V on the position of methylene or fluoro substitution on the parent fentanyl molecule:

Removal of methylene group: alkyl unbranched $<\alpha$ carbonyl (both columns).

Addition of methylene or fluoro group: β-alkyl branched, alicyclic, *meta*- and *para*-fluoro < alkyl unbranched, α-alkyl branched, *ortho* < *meta*- and *para*-methylene (Partisil 10-ODS-3); β-alkyl branched, alicyclic, *meta*-methylene, and *para*-flu-

TABLE VI

COMPARISON OF BINARY MIXTURES AT EQUAL SOLVENT STRENGTH

Solute	*Mobile phase*			*Ref.*
	A	*B*	*C*	
Fentanyl	40% methanol, 60% buffer	28% acetonitrile, 72% buffer	10% THF, 90% buffer	This study Partisil 10-ODS-3
Fentanyl	49% methanol, 51% buffer	25% acetonitrile, 75% buffer	9% THF, 91% buffer	This study PRP-1
Benzene	50% methanol, 50% water	40% acetonitrile, 60% water	37% THF, 63% water	Ref. 6
Methylthio-naphthalene	63% methanol, 37% water	52% acetonitrile, 48% water	39% THF, 61% water	Ref. 4

oro < α-alkyl branched, *ortho*-methylene < alkyl unbranched, *meta*- and *para*-methylene (PRP-1).

Similar trends were found by other authors[1,13,22–24] for different solutes.

Effect of temperature on group contribution

Although our experiments were carried out at 40°C which would tend to reduce

TABLE VII

VARIATION OF GROUP CONTRIBUTION VALUES WITH TEMPERATURE FOR PARTISIL 10-ODS-3 AND PRP-1 COLUMNS

Abbreviations as in Table III.

Compound	*Buf–MeOH (60:40) ODS-3*			*Buf–ACN (75:28) ODS-3*			*Buf–THF (90:10) ODS-3*		
	25°C	*40°C*	*55°C*	*25°C*	*40°C*	*55°C*	*25°C*	*40°C*	*55°C*
1	−0.35	−0.33	−0.32	−0.38	−0.36	−0.37	−0.46	−0.45	−0.45
2	−0.06	−0.06	−0.05	−0.15	−0.14	−0.13	−0.15	−0.14	−0.14
3	0	0	0	0	0	0	0	0	0
4	−0.26	−0.25	−0.23	−0.21	−0.21	−0.21	−0.29	−0.28	−0.28
6	0.20	0.18	0.17	0.18	0.18	0.17	0.28	0.26	0.26
7	0.07	0.06	0.06	0.12	0.11	0.11	0.08	0.08	0.08
11	0.19	0.19	0.18	0.16	0.16	0.16	0.21	0.21	0.20
12	0.27	0.26	0.24	0.22	0.22	0.22	0.31	0.30	0.29
13	0.31	0.29	0.28	0.24	0.24	0.24	0.36	0.34	0.33
17	0.18	0.17	0.16	0.18	0.17	0.17	0.23	0.22	0.21
18	0.27	0.26	0.24	0.22	0.22	0.22	0.33	0.31	0.30
19	0.29	0.27	0.26	0.24	0.23	0.23	0.34	0.33	0.31
22	0.21	0.19	0.19	0.14	0.14	0.15	0.20	0.19	0.18
25	0.02	0.02	0.02	0.09	0.09	0.08	0.14	0.14	0.13
26	0.02	0.02	0	0.08	0.07	0.06	0.16	0.16	0.15

Compound	*Buf–MeOH–ACN (66:20:14) ODS-3*			*Buf–ACN THF (81:14:5) ODS-3*			*Buf–MeOH–THF (75:20:5) ODS-3*		
	25°C	*40°C*	*55°C*	*25°C*	*40°C*	*55°C*	*25°C*	*40°C*	*55°C*
1	−0.38	−0.38	−0.34	−0.44	−0.42	−0.41	−0.41	−0.40	−0.39
2	−0.11	−0.11	−0.10	−0.15	−0.15	−0.14	−0.11	−0.11	−0.11
3	–	–	–	–	–	–	–	–	–
4	−0.24	−0.25	−0.23	−0.25	−0.25	−0.25	−0.26	−0.26	−0.25
6	0.21	0.19	0.18	0.24	0.22	0.22	0.24	0.23	0.21
7	0.10	0.09	0.09	0.11	0.10	0.10	0.07	0.07	0.07
11	0.19	0.19	0.18	0.19	0.19	0.18	0.20	0.19	0.19
12	0.27	0.26	0.25	0.28	0.27	0.26	0.29	0.28	0.26
13	0.31	0.29	0.26	0.32	0.30	0.29	0.33	0.32	0.30
17	0.20	0.19	0.17	0.22	0.20	0.20	0.20	0.19	0.18
18	0.26	0.26	0.23	0.28	0.27	0.26	0.30	0.28	0.27
19	0.28	0.27	0.25	0.30	0.29	0.28	0.31	0.29	0.28
22	0.18	0.18	0.17	0.18	0.16	0.16	0.20	0.18	0.18
25	0.06	0.06	0.05	0.11	0.11	0.10	0.09	0.09	0.08
26	0.06	0.05	0.04	0.11	0.11	0.09	0.10	0.09	0.09

TABLE VII (*continued*)

Compound	*Buf–MeOH–ACN–THF (75:13:9:3) ODS-3*			*Buf–MeOH–ACN–THF (73:16:8:3) PRP-1*		
	25°C	*40°C*	*55°C*	*25°C*	*40°C*	*55°C*
1	−0.41	−0.41	−0.38	−0.52	−0.50	−0.49
2	−0.13	−0.12	−0.12	−0.18	−0.18	−0.17
3	–	–	–	–	–	–
4	−0.26	−0.26	−0.24	−0.33	−0.31	−0.30
6	0.24	0.23	0.21	0.30	0.28	0.26
7	0.10	0.09	0.09	0.12	0.12	0.12
11	0.21	0.21	0.19	0.22	0.21	0.20
12	0.32	0.28	0.27	0.31	0.30	0.29
13	0.33	0.32	0.30	0.35	0.33	0.32
17	0.22	0.20	0.20	0.25	0.24	0.22
18	0.32	0.28	0.27	0.32	0.31	0.29
19	0.31	0.29	0.28	0.34	0.32	0.31
22	0.20	0.18	0.18	0.19	0.18	0.18
25	0.08	0.09	0.07	0.11	0.11	0.10
26	0.09	0.09	0.08	0.10	0.09	0.08

hydrophobic effects, elevated temperature was utilized in order to reduce solvent viscosity and k' values[25]. The effect of temperature on τ was investigated for a limited number of fentanyl homologues and analogues, as indicated in Table VII. In most instances there is only a small decrease in τ (for positive τ values) when temperature is increased. A similar finding, as indicated in Table V, resulted when a PRP-1 column was used with mobile phase containing three organic modifiers. This is in agreement with the reports of other workers[1,2,26].

CONCLUSIONS

Equations relating hydrophobic group contribution to a topological index which is sensitive to bond orders and electronic valences were developed. In addition to providing a means of studying hydrophobic selectivity these equations could be used to predict retention behavior.

For two quite different reversed-phase columns and with mobile phases used for overlapping resolution mapping, hydrophobic selectivity was found to be approximately independent of both mobile phase and stationary phase. Hydrophobic selectivity was shown to depend on the position and type of substitution on the parent fentanyl molecule. In addition, hydrophobic selectivity is found to decrease slightly with temperature.

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