washed with CH_2Cl_2 and the combined organic layers were concentrated. Column chromatography on silica gel using 10% ether/hexane yielded two products identified as 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-phenyl-D-*gluco*-hept-1-enitol (**10**, Ar = Ph) and 3,4,5,7-tetra-*O*-benzyl-2-phenyl-D-*gluco*-hept-1-enitol (**11**, Ar = Ph).

10 (Ar = Ph): ^{1}H NMR (360 MHz) δ 3.75–3.90 (m, 4 H), 4.02 (d br, J = 5 Hz, 1 H), 4.12 (ddd, J = 10 Hz, 5 Hz, 2 Hz, 1 H), 4.50–4.81 (4 AB q, ArCH_2), 5.72 (s, br, 1 H), 7.10–7.40 (m, aromatic H), 7.69 (dm, J = 7 Hz, 2 H).

11 (Ar = Ph): ^{1}H NMR (360 MHz) inter alia δ 5.43 (dd, J = 12 Hz, 4 Hz, 1 H), 6.03 (d, J = 12 Hz, 1 H).

Addition of (4-Methoxyphenyl)mercuric Acetate to 3a. By the procedure described above the corresponding 4-methoxy adducts **10** and **11** were prepared in 13 and 49% yields, respectively.

10 (Ar = 4-OMeC$_6$H$_4$): ^{1}H NMR (360 MHz) δ 3.76 (s, 3 H), 3.75–3.83 (m, 3 H), 3.87 (dd, J = 11 Hz, 2 Hz, 1 H), 4.02 (d br, J = 5 Hz, 1 H), 4.06 (m, 1 H), 4.50–4.82 (m, 8 H), 5.70 (s br, 1 H), 6.76 nd, J = 9 Hz, 2 H), 7.15–7.40 (m, aromatic H), 7.64 (d, J = 9 Hz, 2 H); MS, M$^+$ ion not observed.

11 (Ar = 4-OMeC$_6$H$_4$): ^{1}H NMR (360 MHz) δ 3.00 (d, J = 5 Hz, 1 H), 3.48 (d, AB q, J = 5 Hz, J_{AB} = 10 Hz, 2 H), 3.59 (dd, J = 6 Hz, 3 Hz, 1 H), 3.76 (s, 3 H), 3.77 (m, 1 H), 3.92 (m br, 1 H), 4.19–4.80 (m, 9 H), 5.36 (s br, 1 H), 5.46 (d, J = 2 Hz, 1 H), 6.77 (dm, J = 8 Hz, 2 H), 7.10 (m, 2 H), 7.20–7.40 (m, aromatic); MS, M$^+$ ion not observed.

Addition of (4-Methoxyphenyl)mercuric Acetate to 3c. With **3c** and (4-methoxyphenyl)mercuric acetate the adduct **10** (R = TES, Ar = 4-OMeC$_6$H$_4$) was prepared. Isolation of the open-chain isomer **11** (R = TES, Ar = 4-OMeC$_6$H$_4$) was presumably made difficult because of its hydrolytic instability: ^{1}H NMR (360 MHz) δ 0.65 (m, 24 H), 0.95 (m, 31 H), 3.74 (dd, J = 9 Hz, 2 Hz, 1 H), 3.78–3.87 (m, 2 H), 3.81 (s, 3 H), 3.98 (dd, J = 11 Hz, 2 Hz, 1 H), 4.05 (d, J = 4 Hz, 1 H), 4.32 (ddd, J = 9 Hz, 5 Hz, 2 Hz, 1 H), 5.23 (s, 1 H), 6.81 (d, m, J = 8 Hz, 1 H), 7.60 (dm, J = 8 Hz, 1 H).

Cyclization of 3,4,5,7-Tetra-*O*-benzyl-2-(4-methoxyphenyl)-D-*gluco*-hept-1-enitol (11, R = CH$_2$C$_6$H$_5$, Ar = 4-MeOC$_6$H$_4$). A mixture of 0.123 g of the enitol **11** and 0.090 g of mercuric trifluoroacetate in 3 mL of THF was stirred at 0 °C for 90 min. A check by TLC indicated the reaction to be complete and exceptionally clean. The solution was adjusted to pH 10 with 1 N NaOH, and 0.6 mL of 1 M sodium borohydride in 1 N NaOH was added to the reaction mixture. The solution was stirred for another 1 h at 0 °C, neutralized with 1 M acetic acid, and filtered. The product was extracted into ether. Concentration of the ether extract followed by filtration through a short bed of silica gel yielded the expected product (**12**) with the following characteristic ^{1}H NMR spectrum: ^{1}H NMR (360 MHz) δ 1.62 (s, 3 H), 3.40 (d, J = 10 Hz, 1 H), 3.70–3.90 (m, 5 H), 3.78 (s, 3 H), 4.40–4.95 (m, 8 H), 6.85 (dm, J = 9 Hz, 2 H), 7.07 (m, 2 H), 7.15–7.40 (m, aromatic), 7.52 (d, J = 9 Hz, 1 H).

Carbonyl Reduction by Alcohol Dehydrogenase. A Structure–Activity Study†

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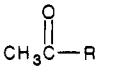
Alcohol dehydrogenase (ADH) catalyzes the oxidation reduction of ethanol as well as many other alcohols and carbonyl compounds:

$$CH_3CH_2OH + NAD \xrightarrow{\text{alcohol dehydrogenase}} CH_3CHO + NADH + H^+ \quad (1)$$

Since the reaction is stereoselective, it has been used to reduce various carbonyl compounds for preparative purposes.[1-3] Although there has been discussion of the structural features that affect the rate of reduction of carbonyl compounds, a large enough data set run under uniform conditions for correlation analysis with substituent constants has not been available. The recent publication by Keinan et al.[3] supplies a good set for analysis.

In undertaking the analysis of their data, we have been guided by our quantitative structure–activity relationships (QSAR) for the inhibition of ADH by a wide variety of inhibitors[4-6] and by a molecular graphics analysis[6] of ADH based on a model constructed from the X-ray crystallographic coordinates of a pyrazole bound to the enzyme.

The active site of ADH is a long narrow cavelike structure with a Zn atom at one end to which electron-rich species may bind. It is to the Zn that the oxygen binds, with the hydrophobic portion of carbonyl compound binding in the long hydrophobic pocket. In the binding of various inhibitors it was found that the hydrophobic and electronic effects of substituents played an important role in the enzyme–ligand interaction. Substituents increasing the electron density on oxygen or nitrogen binding to the Zn increased inhibitor potency. Also an increase in hydrophobicity increased binding. For the present data set in Table I we would also expect steric effects of substituents to play a role in the reduction of the carbonyl group. Hence, one would anticipate having to disentangle electronic, steric, and hydrophobic substituent effects. Normally one likes to have at least five data points/variable in a correlation equation, which would mean 15 for the three variables. In the case of the data in Table I there is so little variation in the electronic effects of the carbon-containing substituents that one could expect a good correlation neglecting electronic effects. A perusal of the results of Keinan et al. reveals that one of the two alkyl groups must be relatively small to obtain reasonable reduction rates. Hence, we have treated only the methyl ketones (I) of which there were, in all, 15. Of these, two



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were inactive [R = CH=C(CH$_3$)$_2$, (CH$_2$)$_3$CH$_3$] and for one [CH$_2$CH$_2$CH=C(CH$_3$)$_2$] the steric parameter E_s is not available. Thus, 12 congeners were suitable for study.

Keinan et al. determined the relative rate of reduction, which is of course composed of two major steps: the formation of the ES complex governed by K_m and the catalytic step (k_{cat}). One would not expect the substituent effect to be the same for these two different enzymic processes, but there is evidence that effects can be additive.[7] In fact we have found that while $1/K_m$ often in-

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Table I. Parameters for the Reduction of $CH_3C(=O)R$ by Alcohol Dehydrogenase

R	log k_{rel}[a] obsd	log k_{rel}[a] calcd	$\lvert\Delta \log k_{rel}\rvert$	log P[b]	E_s[c]
CH_2CH_3	1.08	1.12	0.04	0.26	−1.31
$CH(CH3)_2$	0.48	0.37	0.11	0.57	−1.71
cyclopropyl	−0.10	−0.14	0.04	0.09	−2.21
$CH_2CH_2CH_3$	0.52	0.42	0.10	0.79	−1.60
$(CH_2)_3CH_3$	0.00	0.11	0.11	1.32	−1.63
$CH_2CH(CH_3)_2$	−0.52	−0.63	0.11	1.19	−2.17
$CH(CH_3)CH_2CH_3$	−1.00	−0.89	0.11	1.10	−2.37
$(CH_2)_4CH_3$	−0.05	−0.17	0.12	1.85	−1.64
$(CH_2)_5CH_3$	−0.22	−0.28	0.06	2.37	−1.54
$(CH_2)_6CH_3$	−0.52	−0.60	0.08	2.91	−1.57
$(CH_2)_7CH_3$	−1.00	−0.86	0.14	3.44	−1.57
$(CH_2)_3Cl$	0.18	0.39	0.21	0.50	−1.72

[a] Calculated with eq 2. [b] See ref 8 for calculation procedure. [c] From ref 10.

creases with increased hydrophobic character of the ligand, as does K_i, k_{cat} can be inhibited by the hydrophobic character of the ligand.[7,8] That is, if the substrate is too tightly bound by hydrophobic forces, its desorption after the catalytic step is so slow that the binding of the next molecule of substrate to the active site is inhibited. Hence, in the present case the coefficient with the log P term represents the sum for the adsorption and desorption processes. The same applies to E_s.

An interesting aspect of the work of Keinan et al. is that they employed an unusual alcohol dehydrogenase obtained from *Thermoanaerobium brockii*. This enzyme is extremely thermophilic (stable up to 85 °C) and exhibits high tolerance toward organic solvents.[3]

Results and Discussion

From the data in Table I we have formulated eq 1 and 2.

$$\log k_{rel} = -0.37\ (\pm 0.31) \log P + 0.41\ (\pm 0.53) \quad (1)$$

$n = 12$, $r = 0.642$, $s = 0.496$, $F_{1,10} = 7.02$, $F_{1,10\alpha.025} = 6.94$

$$\log k_{rel} = -0.50\ (\pm 0.09) \log P + 1.50\ (\pm 0.29) E_s + 3.22\ (\pm 0.56) \quad (2)$$

$n = 12$, $r = 0.982$, $s = 0.129$, $F_{1,9} = 139$, $F_{1,9\alpha\cdot001} = 22.9$

In the above expressions log k_{rel} is the relative rate of reduction of methyl ketones I, log P is the calculated[9] octanol–water partition coefficient, and E_s is Taft's steric parameter scaled so that E_s for H = 0.[10] The figures in parentheses are for the construction of the 95% confidence limits, n represents the number of data points, r is the correlation coefficient, s is the standard deviation from the regression, and F is the F statistic. Of the two possible single-variable equations (log P or E_s), the one in log P is slightly more significant ($r = 0.642$ vs. 0.521). Fortunately, there is little collinearity between E_s and log P ($r^2 = 0.087$). Both eq 1 and 2 are highly significant statistically, and eq 2 accounts for 96.4% of the variance in log k_{rel}. Most important, eq 2 makes sense in terms of what we know about the enzyme and its structure–activity relationships.[5,6]

A most interesting aspect of these equations is that the coefficient with log P is negative, revealing that the more hydrophobic R is, the slower the rate of reduction. Our previous studies suggest that for the formation of the ligand–enzyme complex one would expect a positive correlation with hydrophobicity. Therefore, it would seem that for the compounds in Table I the rate-controlling step is that of reduction. The negative hydrophobic term suggests that desorption of the reduced substrate is critical. This is reminescent of the results we have found for emulsin[7] and papain.[8]

Bearing in mind that E_s values for all substituents except H are negative, the positive coefficient with this term in eq 2 shows that steric effects strongly decrease the reduction rate. Of course there are two kinds of steric effects to consider—intra- and intermolecular. However, from our studies with molecular graphics[6] we would not expect the steric effects with the enzyme surface to be significant for the rather linear R groups in Table I. Hence, we believe that E_s is associated with intramolecular effects.

When the magnitude of the coefficient with E_s in eq 2 is considered, it is of interest to compare it with another enzymic process also involving the formation of a tetrahedral carbon intermediate. Equation 3 has been derived

$$\log (k_2/K_m) = 1.51E_s + 0.63\pi + 2.98$$

$$n = 8,\ r = 0.981,\ s = 0.201 \quad (3)$$

for the hydrolysis of $RC(=O)OC_6H_4NO_2$ (II) at pH 7.99 by chymotrypsin.[11] In this expression, π is the hydrophobic substituent constant[9] for R and k_2 is the rate constant for the acylation step in the attack of the Ser OH of chymotrypsin on esters II. The reaction upon which eq 3 is based is not directly comparable to that upon which eq 2 is based, but they are similar and do show similar steric effects.

The fact that a good correlation has been obtained with eq 2 suggests that further experiments to study the electronic effects of the substituents would likely yield good results, but what the value of ρ might be is not clear. For simple binding of an inhibitor, the more electron releasing the substituent the better the binding (i.e., ρ is negative). In the reduction of the ketones, in addition to binding, the transfer of H to the carbon of the carbonyl group must occur. This would be promoted by electron-attracting R groups. Thus, one might expect a negative ρ for the K_m process and a positive ρ for the k_{cat} process. Hence, for further mechanistic studies it would be best to determine K_m and k_{cat}. One could then derive correlation equations for both the binding and the hydride-transfer process.

In terms of the electronic effect of substituents it is noteworthy that when R is $CH{=}C(CH_3)_2$, no reduction occurred. Such a substituent would delocalize the positive charge on the carbonyl carbon as well as make binding tighter. These effects might account for the low activity. When R = $(CH_2)_8CH_3$, the ketone is also unreactive. Keinan et al. speculate that this may be due to the very low solubility of the 11-carbon ketone.

Equation 2 does imply that by making R more hydrophilic with groups such as OH, CN, $SOCH_3$, $CONH_2$, etc., a faster rate of reduction can be achieved.

Registry No. MeCOEt, 78-93-3; MeCOPr-*i*, 563-80-4; MeCOPr, 107-87-9; MeCOBu, 591-78-6; MeCOBu-*i*, 108-10-1; MeCOBu-*sec*, 565-61-7; $MeCO(CH_2)_4Me$, 110-43-0; $MeCO(CH_2)_5Me$, 111-13-7; $MeCO(CH_2)_6Me$, 821-55-6; $MeCO(CH_2)_7Me$, 693-54-9; $MeCO(CH_2)_3Cl$, 5891-21-4; 1-cyclopropylethanone, 765-43-5; alcohol dehydrogenase, 9031-72-5.

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