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AFFINITY, REACTIVITY AND STRUCTURE IN ACETAL FORMATION. II

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The investigation of the relationship between the structure of various alcohols and aldehydes and the rate and extent of the reversible reaction $RCHO + 2R'OH \rightleftharpoons RCH(OR')_2 + H_2O$ has been continued.[1] It has been often assumed that the effect of a given substituent upon the equilibrium point was qualitatively if not quantitatively constant. There would then be certain groups that might be described as "positive" and others as "negative." It has now become evident that this is by no means true. A methyl group, for example, may in one case increase affinity and in another decrease the affinity for the same type of reaction. A methyl group may be more "negative" than a phenyl, a bromine more "positive" than a hydrogen and an hydroxyl more "negative" than a bromine.

This is a result to be expected if the effect of a group in lowering or increasing affinity is considered to be the result of a shift of the electronic structure. The shift resulting from the introduction of a substituent into a compound will depend not only on the character of the substituent but upon the character of the substituted compound, that is, upon the particular electronic system that is acted upon. The *effect* of the shift upon affinity will be dependent upon a third factor, that is, upon whether the shift has produced an electronic configuration which requires *more* or *less* energy to get the electrons (that is, their orbits) into a position where a new linkage may be formed with the particular reactant involved. It appears that a given substituent does not bring about simply a dislocation of electron pairs towards or away from the reactive end of the molecule but that it modifies the whole electronic architecture of the molecule. It is evident that the positivity or negativity of a group may not be defined except for the reactions of specific compounds.

The experimental results are summarized in Table I. For the sake of convenience the aldehydes are regarded as beta substitution products of acetaldehyde, a quantitative measure of the effect of the substituent being given in the third column of the table under $-RT \ln K_e$. The value of the expression $-RT \ln K$ would be that of the decrease in free energy ΔF if the activities of the reactants were equivalent to the analytically determined concentrations. Since for the present the activities are not known, it seems preferable not to consider the decrease in free energy to be equal to the values of $-RT \ln K$ but to refer to them as "affinity

[1] Adkins and Adams, THIS JOURNAL, **47**, 1368 (1925).

values." It is possible that the absolute values of $-RT \ln K$ are of little significance,[2] and that even the relative values vary in different solvents.

The equilibrium point was approached from both sides in the case of aldehydes 4, 5, 7, 8, 12 and 15. The data for aldehydes 1, 2, 3, 6, 10, 11, 12 and 15 have not been previously published. Data on aldehydes 4, 5, 7, 8 and 14 were published by Adams and Adkins but the experimental work has been repeated and corrected values are given here. The new values are of the same order as those previously published except in the case of heptaldehyde. The equilibrium constants for aldehydes 9 and 13 were recalculated from Adams' data and are given here for the sake of ready comparison. Aldehydes 1, 2, 3, 6, 10 and 11 have never been prepared in a state of purity so that the equilibrium could not be obtained from the synthetic side.

TABLE I

EFFECT OF BETA SUBSTITUTION IN ACETALDEHYDE UPON AFFINITY IN ACETAL FORMATION

No.	Aldehyde	β-substituent	K	$-RT \ln K$
1.	Amino-acetaldehyde	NH_2HCl—	0.1340	1190 ± 200
2.	Bromo-acetaldehyde	Br—	.1121	1300 ± 200
3.	Cyano-acetaldehyde	CN—	.1070	1320 ± 200
4.	Heptaldehyde	C_6H_{11}—	.0970	1380 ± 30
5.	Propionaldehyde	CH_3—	.0782	1510 ± 30
6.	Imino-acetaldehyde	—NH(HCl)—	.0770[a]	1520 ± 200
7.	Acetaldehyde	H—	.0744[b]	1540 ± 30
8.	Butyraldehyde	C_2H_5—	.0691	1580 ± 30
9.	*iso*Butyraldehyde	$(CH_3)_2$	.0355	1980 ± 30
10.	β-Chloropropion-aldehyde	CH_2Cl—	.0294	2090 ± 200
11.	Glycolic aldehyde	HO—	.0202	2300 ± 200
12.	Acrolein	CH_2=	.00455	3270 ± 30
13.	Benzaldehyde	...	.00163	3810 ± 30
14.	Cinnamaldehyde	C_6H_5CH=	.00089	4160 ± 30
15.	Crotonaldehyde	CH_3CH=	.00063	4360 ± 30

[a] The calculations are based on the assumption of the hydrolysis of both acetal groups.

[b] The authors are indebted to A. E. Broderick for the value for diethyl acetal and for other valuable assistance.

The equilibrium points for these six aldehydes were determined by the colorimetric method so that the values for $RT \ln K$ should be read ±200. The analytical method used for the determination of the other aldehydes should give a value accurate to within ± 30 units.

From an inspection of the table it becomes evident that the normal chain aliphatic aldehydes (Nos. 4, 5, 7 and 8) occupy, within a relatively narrow range, nearly the same position and are very high in the scale, whereas *iso*butyraldehyde (No. 9), with a branched chain, is decidedly lower. The hydrochloride of amino-acetaldehyde shows a high affinity

[2] See Conant, THIS JOURNAL, 49, 293 (1927).

value, as would be expected from the positive nature of the amino group. Bromo-acetaldehyde and cyano-acetaldehyde are practically alike as one would expect but that both should show a slightly greater affinity than acetaldehyde is indeed striking, for both the bromo and cyano groups are commonly considered negative and one would, therefore, expect them to have a marked depressing effect. Glycolic aldehyde, on the other hand, shows a marked lowering of affinity as compared with acetaldehyde. While a halogen on the alpha carbon of acetaldehyde is "positive" in its effect, the same is not true of beta-halogenated propionaldehyde, for β-chloropropionaldehyde is decidedly lower in affinity than is propionaldehyde. These results seem to conflict with those obtained by Wegscheider[3] and Derick[4] who studied the effect of halogen substitution on the dissociation constants of saturated aliphatic acids.

In 2,3 unsaturated aldehydes the affinity is much lower than in the saturated aldehydes. Acrolein drops almost to the level of benzaldehyde, and if a methyl or phenyl group is substituted for hydrogen on the beta carbon of acrolein a further lowering in affinity is observed, the alkyl having an even greater effect than the aryl group. That the phenyl group should exercise such influence is not surprising when one compares the relative positions of acetaldehyde and benzaldehyde; but the affinity value for crotonaldehyde indicates that the effect of a methyl in an unsaturated aldehyde is very different from that in a saturated one.

Experimental Method

In general the procedure of Adams was followed, known amounts of reactants being mixed in a dry Pyrex test-tube of suitable size and the tube closed with a stopper bearing a sampling device. The latter was made by sealing a 5cc. pipet to the single arm of a three-way stopcock. One of the double arms was extended to reach the bottom of the reaction tube while the other was lengthened and bent to permit exactly 5 cc. of the solution to be delivered into a suitable receiver. The concentration of the catalyst in the synthetic reactions was usually that used by Adams, while in the slower hydrolytic reactions the concentration was five or ten times as great. The reactions were allowed to proceed at 25°. In those cases where the aldehyde was available both the synthetic and hydrolytic reaction were carried out. In the other cases the hydrolysis was carried out at two different concentrations.

Preparation and Purification of Materials

Ethyl alcohol, acetaldehyde and ethyl acetal were purified as described in previous papers.[5] The ethyl acetals of propionaldehyde, butyraldehyde and heptaldehyde were

[3] Wegscheider, *Monatsh.*, **23**, 289 (1902).

[4] Derick, THIS JOURNAL, **33**, 1152 (1911).

[5] (a) Child and Adkins, *ibid.*, **45**, 3013 (1923). (b) Adkins and Nissen, *ibid.*, **44**, 2749 (1922).

prepared by Nissen's method. The diethylpropional had a density (referred to water at 4°) of 0.8232, and the diethylbutyral, of 0.8417 at 25°. Butyraldehyde was purified through the bisulfite compound, the vapors being dried over calcium chloride as in the purification of acetaldehyde and finally fractionated under reduced pressure. It boiled below 12.5° at 60 to 80 mm. Its density at 25° was 0.7988 and its refractive index (Abbé) at 25° was 1.3750. The propionaldehyde boiled 46.5 to 47.4° at 740 mm. The cinnamic aldehyde boiled at 108–109° at 35 mm. Its density at 25° was 1.045. Heptaldehyde was purified through the bisulfite addition compound and by the hydrolysis of diethylheptal. The final step in purification was a distillation carried out so slowly at 30–34° at 4 mm. that there was no noticeable ebullition. The density at 25° was 0.815 and the refractive index (Abbé) at 25° was 1.4077.

The Diethyl Acetal of Bromo-acetaldehyde was prepared by a modification of the method of Pinner[6] and Fischer.[7] One mole of bromine was added from a dropping funnel, during 30 to 45 minutes, to a well agitated mixture of one mole of diethyl acetal and 55 g. of precipitated calcium carbonate. The reaction mixture was kept in an ice-bath but the bromination proceeded best if the temperature was allowed to rise as much as possible without allowing the loss of the acetal, namely, to about 10°. The mixture was allowed to stand for 8 to 24 hours and enough steam introduced to dissolve the salts. The crude oil was then separated and placed over potassium carbonate. The aqueous layers from several brominations were steam distilled and the acetal so obtained was added to the crude oil, which was then washed with potassium carbonate solution until free from acid and dried for at least 12 hours over fresh potassium carbonate. The product was then fractionated. From 246 to 332 g. of the bromo-acetal boiling at 167–170° was obtained from 472 g. (4 moles) of acetal. This slightly colored product may be purified by distilling at 3 mm. pressure, shaking for 45 minutes with granular potassium carbonate and distilling at 48–49° (3 mm.). The density at 25° is 1.28. The pure acetal after a few hours becomes colored, and black after several days. The crude acetal, that is, the material boiling at 167–170°, was kept for months with little loss. It is a powerful lachrymator.

The Cyano-acetal was prepared by a modification of the method of Wohl.[8] One hundred and thirty-two g. of bromo-acetal, 86.5 g. of potassium cyanide, 11 g. of potassium iodide, 300 cc. of alcohol and 150 cc. of water were gently refluxed for 72 hours in an oil-bath. The reaction mixture was steam distilled, the first portions of the distillate being diluted with water to throw out the acetal. After drying over granular potassium carbonate the crude product was fractionated at 5mm. pressure, the compound boiling at 52–54°. At 15 mm. the boiling point is 55–57°; higher pressures result in considerable decomposition. The water-white acetal, density 1.255 at 24°, remains colorless if sealed in glass but becomes colored if allowed to stand in air. Such colored acetal should be allowed to stand with potassium hydroxide for an hour and be redistilled at 5 mm. pressure.

The Acetal of Glycolic Aldehyde was prepared by a modification of the method of Pinner.[6] A mixture of 66 g. of bromo-acetal, 37 g. of potassium hydroxide and 300 cc. of 95% alcohol was gently refluxed for 72 hours. The alcohol was distilled and the residue shaken with water. The oily layer was separated and the water layer extracted with ether. The oily layer was added to the ether and the whole dried over potassium hydroxide for 12 hours. The product was fractionated, that portion boiling at 163–167° being the desired product. This was fractionated at 8 mm., boiling at 57–58°; or 49–51° at 3–4 mm. The yields were from 40 to 60% and the density at 24° was 0.888.

[6] Pinner, *Ber.*, **5**, 149 (1872).

[7] Fischer, *Ber.*, **25**, 2551 (1892).

[8] Wohl, *Ber.*, **39**, 1952 (1906).

Acetal Amine (Amino Acetal) and Diacetal Amine were obtained by a modification of the method of Wohl[9] and Wolff.[10] Absolute ethanol was saturated at 0° with anhydrous ammonia and for each 7 g. of alcoholic ammonia 1 g. of bromo-acetal was used. The mixture was then sealed in tubes and heated at the temperature of boiling toluene for 18–24 hours. Any solid that may have formed was filtered off and the alcohol distilled from the filtrate. The residue from the distillation and the solid first obtained were combined, taken up in water and treated with alkali, whereupon an insoluble layer collected on the surface of the liquid. This was drawn off, the aqueous layer extracted several times with ether, the ethereal extract and the product first obtained were combined and dried over solid potassium hydroxide for at least 12 hours. The ether was distilled off and the residue repeatedly fractionated under reduced pressures. Fractions boiling at 52–53° at 2 mm. and at 127–130° at 3 mm. were obtained. The compound of lower boiling point had a density at 25° of 0.9161 and a refractive index (Abbé) of 1.4120. Analysis for nitrogen by combustion showed:

Anal. Calcd. for $H_2NCH_2CH(OC_2H_5)_2$: N, 10.52. Found: 10.88.

The compound of higher boiling point had a density of 0.9541 at 25° and a refractive index of 1.4210.

Anal. Calcd. for $HN(CH_2CH(OC_2H_5)_2)_2$: N, 5.62. Found: 5.96.

Crotonic Aldehyde was prepared from aldol by slowly distilling it at ordinary pressures through a 30cm. Vigreaux fractionating column. The unsaturated aldehyde was obtained from the distillate by salting out with calcium chloride, drying and redistilling at ordinary pressures; b. p., 101–104°.

Acrolein.—The method used for the preparation of acrolein has been described by us in "Organic Syntheses."[11] The product boiled at 52.5 to 53.5° and had a density at 0° of 0.859 and at 25° of 0.8377.

The Acetals of Acrolein and Crotonic Aldehyde and the Acetal of β-Chloropropionaldehyde were prepared by the method of Witzemann[12] and of Evans and Hass.[13] The acetal of β-chloropropionaldehyde, having a boiling point at 3–4 mm. of 47–50° and a density of 0.983 at 25°, was obtained in a yield of 41%. The β-chlorobutyraldehyde acetal used as an intermediate in the preparation of the acetal of crotonic aldehyde boiled at 70–90° at 40–45 mm. pressure. The acetal of crotonic aldehyde boiled at 146–148° at 740 mm. and at 33–34° at 2.5 mm. The yields of the acetal on the basis of crotonaldehyde used were only 4–7%. The density of crotonic aldehyde acetal at 25° was 0.846.

Analytical Determination of the Concentration of Reactants at the Equilibrium Point.—The concentrations at equilibrium of acetaldehyde, heptaldehyde, butyraldehyde, propionaldehyde, cinnamic aldehyde and crotonic aldehyde were determined by the modified Seyewitz-Bardin method as described in previous papers. Numerous titrations of weighed amounts of the purified aldehydes showed that under the conditions present in the titrations one mole of butyraldehyde reacted with 0.95 mole of bisulfite, one mole of heptaldehyde with 0.90 mole of bisulfite, one mole of crotonic aldehyde with 1.85 moles of bisulfite and one mole of cinnamic alde-

[9] Wohl, *Ber.*, **21**, 617 (1888).

[10] Wolff, *Ber.*, **21**, 1481 (1888).

[11] "Organic Syntheses," **6**, 1, John Wiley and Sons, Inc., New York City, **1926**.

[12] Witzemann, THIS JOURNAL, **36**, 1909 (1914).

[13] Evans and Hass, *ibid.*, **48**, 2703 (1926).

hyde with 1.84 moles of bisulfite. It was demonstrated that the alkene linkage, in the acetals of the two last-mentioned aldehydes, did not react with the sulfite under the conditions of titration. The above values were used in calculating from the titration values the amounts of the aldehydes present.

The Seyewitz-Bardin method apparently cannot be used for the determination of acrolein, glycolic aldehyde, cyano-acetaldehyde, bromo-acetaldehyde, β-chloropropionaldehyde, amino- and imino-acetaldehydes. A long and fruitless search was made for a method whereby these aldehydes could be determined in the presence of their acetals. Finally it was found necessary to determine the extent of the reaction by determining the amount of water present in the equilibrium mixture. This was done by using cobaltous chloride in a colorimetric method. An accuracy better than 5% cannot be claimed for this method. Results obtained in this way agreed with the sulfite titration method when the latter could be used, as with acetaldehyde and heptaldehyde.

The pink crystals of cobaltous chloride were dehydrated by heating over a gas flame. A saturated solution of the dried salt was made in absolute alcohol, and three to five drops of this solution (depending on the amount of water present) was used as an indicator for each 5 cc. of solution. It was found that the color of the cobaltous chloride was a function not only of the amount of water but also of the amount of acetal present, but the color was not influenced by the free aldehyde, as with acetaldehyde, heptaldehyde and acrolein.

The amount of water at equilibrium was determined by comparing a 5cc. sample of the reaction mixture containing indicator with a set of standards which were prepared in the following way.

In a series of test-tubes previously cleaned and dried were put equimolecular amounts of water and of the acetal under investigation; the volume of each standard was made up to 5 cc. with absolute alcohol and the cobalt chloride indicator added. By comparing the color of the sample with that of the standards the amount of water in the equilibrium mixture could be determined. Since the amounts of acetal and water for each standard were small, separate solutions containing a known weight of acetal in absolute alcohol (Solution I) and a known weight of water in absolute alcohol (Solution II) were made up in volumetric flasks, and from these the necessary amounts of each were used and absolute alcohol then added to each tube to make the total contents equal to 5 cc.

To illustrate the use of this method an actual case will be given in detail. In a reaction tube were placed

7.255 g.	Chloropropylal	0.0435 mole	7.36 cc.
0.785 g.	Water	.0436 mole	0.79 cc.
23.04 g.	Ethanol-catalyst (HCl)	.479 mole	28.10 cc.
			36.25 cc.

Several days later standards were prepared by using ordinary test-tubes, previously thoroughly cleaned and dried, and putting into them the indicated amounts of Solutions I and II. Solution I contained 10.014 g. of β-chloropropionaldehyde acetal and was made up to 25 cc. with absolute alcohol; Solution II contained 4.346 g. of water and was diluted with absolute alcohol to 50 cc.

No.	G., water	Cc., soln. II	G., acetal	Cc., sol. I	Cc., abs. alc.
1	0.0870	1.00	0.885	2.01	1.96
2	.0521	0.60	.488	1.20	3.20
3	.0261	.30	.242	0.64	4.06
4	.0191	.22	.177	.44	4.34
5	.0174	.20	.161	.40	4.40

A 5cc. sample was withdrawn from the reaction tube and to each of the standards and sample 3 drops of the cobalt chloride indicator was added. A color comparison of the sample with the standards showed it to be like 1. The next day a new set of standards in the vicinity of 1 was made but with less water variation, and in this way it was found that the acetal-equilibrium mixture corresponded to the standard containing 0.0912 g. of water per 5 cc. Thus the amount of unhydrolyzed acetal was found to be 84%.

Calculation of Equilibrium Constants and Affinity Values

The values for K_e listed in Table I are mean values and the manner in which they were obtained, especially where the equilibrium point was determined colorimetrically, is illustrated in the case of glycolic aldehyde. The extreme values of K_e for this aldehyde were found to be 0.0150 and 0.0272, giving for $-RT \ln K$ 2480 units and 2140 units, respectively, of which the average is 2310 units, corresponding to $K_e = 0.0202$; and $-RT \ln K$ becomes 2300 ± 180 units. K_e and $-RT \ln K$ were obtained in a similar manner for other aldehydes.

$$-RT \ln K = 1.9885 \times 298 \times 2.3026 \times \log_{10} k$$

Summary

The equilibrium constants for the reaction of ethanol with amino-acetaldehyde, bromo-acetaldehyde, cyano-acetaldehyde, imino-acetaldehyde, β-chloropropionaldehyde, glycolic aldehyde, acrolein and crotonic aldehyde, heptaldehyde, propionaldehyde, acetaldehyde, butyraldehyde and cinnamaldehyde have been given.

The values of $-RT \ln K$ have been calculated and used as the basis for a comparison of the relative affinity manifested by the various aldehydes in the acetal reaction.

It has been noted that the effect of a given substituent upon affinity in the acetal reaction is neither quantitatively nor qualitatively constant. The same substituent in some cases increases and in others decreases affinity.

Methods for synthesis of several acetals are given and some of their physical constants have been determined.

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THE OSMOMETRIC METHOD OF DETERMINING THE MOLECULAR WEIGHTS OF PROTEINS

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In a recent paper Svedberg and Nichols[2] state that the osmotic pressure method of measuring the molecular weights of proteins is rendered quite uncertain by the Donnan equilibrium. Svedberg and Fåhraeus[3] stated that conflicting results, obtained by different investigators of the molecular weight of hemoglobin, were partly due to the difficulty of measuring osmotic pressures against semipermeable membranes and partly due to the Donnan effect.

An explanation of these difficulties has been suggested.[4] The conflicting results referred to by Svedberg had been obtained with hemoglobin equilibrated with water of unknown hydrogen-ion concentration. New measurements[4] showed that deviations from the isoelectric point led to abnormally high osmotic pressures on account of the excess of ions inside the membrane.

Unfortunately, Svedberg and Fåhraeus did not refer to any of the papers on the osmotic pressure of hemoglobin published in 1924 and 1925, which showed that the membranes gave true equilibria,[4,5,6] and the molecular weights corrected for the ion distribution effects were the same as the figure they obtained by the ultra-centrifugal method, namely, 62,000–71,000 for purified horse hemoglobin.

The osmometric data for the hemoglobins of various species have been given in the papers referred to as follows: hemoglobin of man, the horse and the sheep (solvent $N/10$ NaCl, etc.);[4] hemoglobin of man and the ox (solvent salts of red corpuscle);[6] hemoglobin of the horse and the sheep (solvent, distilled water).[4] All the molecular weights agreed to within 10% of 66,800, which is four times the equivalent calculated from iron analyses.

The agreement of the results with different salt solutions affords a check on the accuracy of the membrane equilibrium corrections in the case of hemoglobin. The empirical correction formula[5] which was pro-

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[2] Svedberg and Nichols, THIS JOURNAL, **48**, 3081 (1926).

[3] Svedberg and Fåhraeus, *ibid.*, **48**, 430 (1926).

[4] Adair, *Proc. Roy. Soc.* (*London*), **109A**, 292 (1925).

[5] Adair, *Proc. Camb. Phil. Soc.* (*Biol.*), 1, 75 (1924).

[6] Adair, *Proc. Roy. Soc.* (*London*), **108A**, 627 (1925).