468. *Linear Free-energy Relations in the Steroid Series. Basic Strengths of Aminocholestanes.*

By C. W. Bird and R. C. Cookson.

Equatorial aminocholestanes are generally more basic than their axial epimers. Because of interference with the 15-methylene group the 7β-dimethylamine is abnormally weak.[1] A plot of the pK_a of the equatorial amines, cyclohexylamine and 2α-, 3β-, 6α-, and 7β-aminocholestane, against the logarithm of the rate of oxidation of the corresponding alcohol with chromic acid gives a straight line. The points for the axial 3α- and 7α-positions fall on a separate but parallel line. The line connecting points for the pair of axial positions opposed to the 10-methyl group, 2β and 6β, is also parallel but even further displaced.[2]

In order to clarify the influence of hindrance to solvation of ions on acid–base equilibria [3] we required a series of epimeric amines attached to a rigid, non-polar framework of known conformation. For the purpose we selected the aminocholestanes.

Reduction of the oximes of the various cholestanones gave in each case a mixture of the two epimeric amines, more equatorial amine from sodium and alcohol, more axial amine from lithium aluminium hydride. Methylation of the primary amines was effected with formaldehyde and formic acid. The bases were purified as their hydrochlorides or hydrobromides (most of which were soluble in chloroform and some even in benzene).

The problem of selecting an aqueous solvent in which all the bases and their salts were sufficiently soluble for determination of their dissociation constants was solved by using 50% t-butyl alcohol. Measurements, made with a glass electrode and calomel standard, were reproducible within $\pm 0{\cdot}03$ unit of pK_a. Although the values no doubt differ from the true thermodynamic constants, deviations are probably roughly constant throughout the series. To compare the solvent with better documented systems, such as ethanol–water, Fig. 1 shows the apparent pK_a of cyclohexylamine in mixtures of water and t-butyl alcohol of varying composition, together with the values recorded [4] for methylamine in mixtures of ethanol and water. On a molar scale t-butyl alcohol seems to be more effective than ethanol in breaking down the quasicrystalline structure of water.

Since in general an ion will be more heavily hydrated than a neutral molecule, a decrease in solvation of a protonated base due to shielding by unsolvated hydrocarbon groups will cause a fall in basic strength. Although the amines are undoubtedly solvated in aqueous solution, the heat of hydration of the protonated ammonium ions is probably at least 70—80 kcal./mole greater,[5] so that one is justified in concentrating attention on changes in hydration of the ions. The pK_a values of the pairs of epimeric aminocholestanes listed in Tables 1 and 2 justify the resulting expectation that an axial amine, being usually less accessible to solvation when protonated, should be a weaker base than its equatorial epimer. In this system the only polar effects within the molecule will be those from the negligible C–H dipoles and the inductive effect, which will be the same for

[1] Preliminary note: Bird and Cookson, *Chem. and Ind.*, 1955, 1479.
[2] For full details see Bird, Ph.D. Thesis, London, 1957.
[3] Wepster, *Rec. Trav. chim.*, 1957, **76**, 357.
[4] Gutbezahl and Grunwald, *J. Amer. Chem. Soc.*, 1953, **75**, 559, 565.
[5] Briegleb, *Z. Elektrochem.*, 1949, **53**, 350.

each of a pair of epimers and very nearly the same for the 2- and 3-amines and for the 6- and 7-amines.

In general, the more hindered the position on the cholestane nucleus, the greater the difference between the basicity of the epimers, in the increasing order, 3, 2, 7, 6. Even

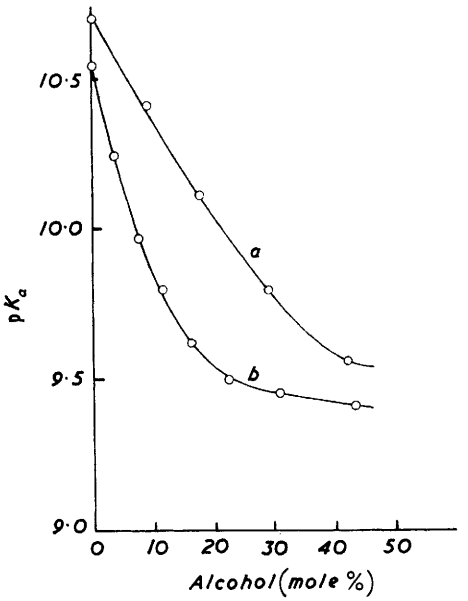


FIG. 1.
(*a*) *Methylamine in ethanol.*
(*b*) *Cyclohexylamine in t-butyl alcohol.*

in our small range of bases, there are however some exceptions to the general rule: it can perhaps be applied safely, at least in our solvent, only to epimeric cyclohexylamines in which the axial epimer is hindered by at least one additional non-polar, axial 3-substituent. Thus the 3-amino-steroids do not conform to the rule: 3α-aminocholestane is stronger than

TABLE 1. *Primary amines.*

Compound	Conformation	pK_a	Compound	Conformation	pK_a
Cyclohexylamine	(eq)	9·71	6α-Aminocholestane	eq	8·99
2α-Aminocholestane	ex	9·25	6β-Aminocholestane	ax	8·38
2β-Aminocholestane	ax	8·99	7α-Aminocholestane	ax	8·21
3α-Aminocholestane	ax	9·42	7β-Aminocholestane	eq	8·55
3β-Aminocholestane	eq	9·30			

TABLE 2. *Tertiary amines.*

Compound	Conformation	pK_a	Compound	Conformation	pK_a
Dimethylcyclohexylamine	(eq)	8·81	3α-Dimethylaminocholest-5-ene	ax	8·53
2α-Dimethylaminocholestane ...	eq	8·38	3β-Dimethylaminocholest-5-ene	eq	8·18
2β-Dimethylaminocholestane ...	ax	8·09	3α-Dimethylaminocoprostane ...	eq	8·36
3α-Dimethylaminocholestane ...	ax	8·32	3β-Dimethylaminocoprostane ...	ax	8·40
3β-Dimethylaminocholestane ...	eq	8·55			
6α-Dimethylaminocholestane ...	eq	8·10			
6β-Dimethylaminocholestane ...	ax	7·56			
7α-Dimethylaminocholestane ...	ax	7·48			
7β-Dimethylaminocholestane ...	eq	6·69			

its equatorial epimer, although the normal relative basicities hold when the amines are methylated; the accuracy of our measurements does not distinguish between the basicities of the two 3-dimethylaminocoprostanes; the axial epimer is the stronger of the two 3-dimethylaminocholest-5-enes. The presence of unsaturation near the basic centre renders the last, of course, a special case. For example, the equatorial amine might be

made less basic by the C ⟶ C= dipole, or the axial salt might be stabilised by hydrogen-bonding to the double bond, either direct or through a water molecule. $C_{(3)}$ is also the most easily distorted position on the cholestane nucleus.

The 7-dimethylaminocholestanes present a particularly interesting anomaly. Although 7α-aminocholestane is, as expected, a weaker base than its equatorial epimer, 7α-dimethylaminocholestane is stronger than 7β-dimethylaminocholestane. In other words, whereas methylation of cyclohexylamine and the other seven aminocholestanes causes an average fall in pK_a of 0·9 (with extreme values of 0·7 and 1·1), methylation of 7β-aminocholestane lowers the pK_a by 1·9, yielding much the weakest of the whole series of bases. The most stable conformation of the cyclohexyldimethylammonium ion would be expected to be that with the $^+$N–H bond parallel to the axial bonds and the hydrogen atom strongly bonded to the solvent. In 7β-dimethylaminocholestane the 15-methylene group makes that conformation impossible and renders the hydrogen atom inaccessible to the solvent.* One can see that in a model the axial 2β- and even 6β-dimethylamines, though hindered, are more accessible, and they are in fact not abnormally weak.

In a mixture of water and t-butyl alcohol the ammonium ions will presumably be surrounded by a primary solvation shell of water molecules only, succeeded by the secondary shell which may well also consist mainly of water molecules.[6] Solvation will, of course, be centred on the positive nitrogen atom, and the projection of an unsolvated hydrocarbon residue through the " iceberg " [7] frozen around that atom will reduce solvation and weaken the base. Now the strengths of the equatorial amines decrease in the order cyclohexylamine > 3β- > 2α- > 6α- > 7β-aminocholestane. In the first three bases at least the inductive effects and the usually recognised repulsions between unbound atoms are almost identical, so that the decreasing basicity can be attributed to increasing disruption of the solvation shell: a charge 5 Å or more from the nitrogen atom causes an appreciable change in basicity.

In a paper [8] that appeared after our work was finished Hall showed that the pK_a values of unhindered primary amines varied linearly with Taft's σ* values for substituents. Plots of the pK_a's of secondary and tertiary amines against the sum of the substituents' σ* values also gave straight lines, but points for each class of amine (primary, secondary, tertiary) lay on separate lines. Branching of the chain made a primary or secondary amine weaker than predicted from the polar σ* values, although it did not affect the strength of tertiary amines. Hall therefore concluded that tertiary amines are much less susceptible to hindrance to solvation than other amines. Thus 1,2,2,6,6-pentamethylpiperidine [9] is the most basic of all tertiary amines. The dramatic fall in pK_a of 7β-dimethylaminocholestane shows, however, that in suitable circumstances tertiary amines are more weakened by hindrance than are primary.

Comparison [10] of the equilibrium constants (K) for the aminocholestanes with the rate constants (k) for oxidation of the corresponding alcohols to ketones by chromic acid [11] yields some interesting regularities. In Fig. 2, log K (=pK_a) for the primary amines is plotted against log k for oxidation of the corresponding alcohols. A similar plot for the tertiary amines is shown in Fig. 3. Although there is an almost perfect linear relation for the equatorial compounds, the points for the axial epimers do not lie on the same, or on any other single, straight line. But lines through the pairs 3α, 7α, and 2β, 6β are almost

* For rather similar reasons 7β-cholestanyl benzoate is hydrolysed faster than its epimer (Barton and Rosenfelder, *J.*, 1951, 1048).

[6] For a review of ionic solvation in water see Feates and Ives, *J.*, 1956, 2798; also Bockris, " Modern Aspects of Electrochemistry," Butterworths, London, 1954, p. 47.
[7] Frank, *J. Chem. Phys.*, 1945, **13**, 378, 393, 507.
[8] Hall, *J. Amer. Chem. Soc.*, 1957, **79**, 5441.
[9] Hall, *J. Amer. Chem. Soc.*, 1957, **79**, 5444.
[10] For a review of linear free-energy relations see Taft in " Steric Effects in Organic Chemistry," ed. Newman, Wiley, New York, 1956.
[11] Schreiber and Eschenmoser, *Helv. Chim. Acta*, 1955, **38**, 1529.

parallel with the single equatorial line. It must be significant that all the points on any one line refer to substituents in similar environments: the first line accommodates equatorial substituents, the second axial substituents opposed only to axial hydrogen atoms, and the third axial substituents opposed to an axial methyl group.

Schreiber and Eschenmoser [11] pointed out that the rate of oxidation of the various secondary alcohols by chromic acid paralleled the intramolecular compression of their hydroxyl groups, very plausibly attributing the trend to steric acceleration: if the rate of formation of the intermediate chromate ester and its equilibrium concentration are not important, the more compressed the hydroxyl group the less will its energy be below that of the transition state, where the carbon atom is becoming trigonal. Whether the proton

FIG. 2. *Plot of* pK_a *for* $-NH_2$ *against* log k (CrO_3).

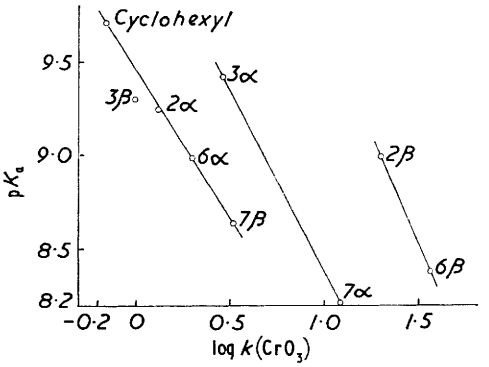
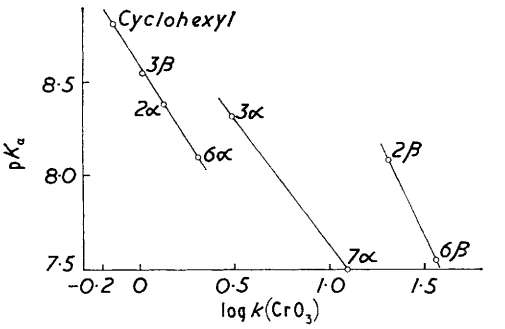


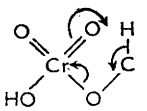
FIG. 3. *Plot of* pK_a *for* $-NMe_2$ *against* log k (CrO_3).



eliminated is pulled off by a solvent molecule or by the departing chromate [12] does not affect the essential argument.

On the other hand, we have attributed changes in basicity of the amines mainly to changes in solvation of the ammonium ions. So we have a remarkably linear relation between a rate controlled by intramolecular forces (steric acceleration) and an equilibrium controlled by intermolecular forces (solvation)—at first sight perhaps a surprising situation. One should at least consider the possibility that the two sets of data share a more obviously common origin, and are either both dictated by intramolecular repulsion or both by changes in solvation energy.

For reasons that have been given,[2,3,13] we reject the possibility that the hindered amines are weaker because the effective size of ^+NH is substantially greater than that of N with an electron pair. The possibility remains, though, that the rates of oxidation by chromic acid are also mainly controlled by solvation. For example, if the chromate ester were more solvated than the transition state for its breakdown to ketone, then hindrance to solvation would increase the rate. Or again, if the oxidation proceeds through a cyclic transition state (A), the more hindered the chromate ester is to external solvation, the faster it will remove the proton from carbon.



(A)

However, in the absence of knowledge about the detailed mechanism of oxidation, all we can say is that there is a parallel between the weakness of a base and the rate of oxidation of the corresponding alcohol, and that both are due to pressure on the substituent or its associated solvent molecules.

The only other reactions in this series for which quantitative data are available are the reductions of ketones [14] and of oximes by lithium aluminium hydride, where the yields of

[12] Westheimer *et al.*, *J. Amer. Chem. Soc.*, 1952, **74**, 4387 and previous papers; Rocek and Krupicka, *Coll. Czech. Chem. Comm.*, 1958, **23**, 2068; Kwart and Francis, *J. Amer. Chem. Soc.*, 1959, **81**, 2116.
[13] Aroney and Le Fèvre, *J.*, 1958, 3002.
[14] Dauben, Blanz, Jiu, and Nicheli, *J. Amer. Chem. Soc.*, 1956, **78**, 3752.

epimers are known. The proportion (Q) of the two epimers formed by reduction of the ketone will be the ratio of their rates of formation. A plot of the log of the ratio of the rates of oxidation of the alcohols with chromic acid against the log of the ratio of the amounts of the epimeric alcohols formed by reduction again gives two parallel straight lines (Fig. 4). The line joining the points for 2- and 6-compounds in a similar plot for reduction of oximes is also widely spaced from, but parallel to, the line joining 3- and 7-compounds.

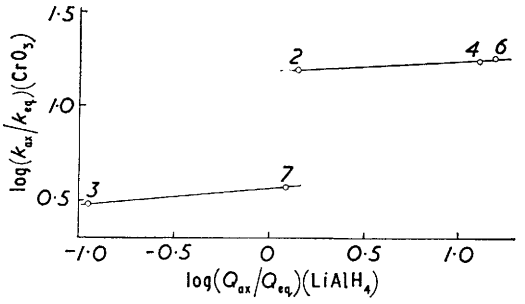


FIG. 4. *Plot of* log (k_{ax}/k_{eq}) (CrO_3) *against* log (Q_{ax}/Q_{eq}) ($LiAlH_4$) *for ketones.*

EXPERIMENTAL

M. p.s are corrected. Rotations were measured for $CHCl_3$ solutions. Solutions for chromatography were prepared in light petroleum (b. p. 40—60°), and chromatographed on Spence's Grade H alumina with solvents of increasing polarity. Axial amines were always eluted before their equatorial epimers. It was impossible to obtain satisfactory analyses for several compounds.

Reduction of Oximes.—Reduction with sodium and pentyl alcohol gave the equatorial aminocholestane.[15] Reduction with lithium aluminium hydride was carried out in boiling ether for 1—4 days. The excess of hydride was then decomposed and enough water was added to coagulate the aluminium hydroxide, which was removed and washed twice with chloroform. The residue from evaporation of the solvents was taken up in dry ether, from which the bases were precipitated as their hydrochlorides. The regenerated bases were chromatographed quantitatively, to give the following proportions of axial amine in the epimeric mixture: 3-, 67%; 2-, 72·5%; 6-, 90%; 7-, 90%.

The primary amines were methylated with a hot mixture of formaldehyde and formic acid.[15]

2α-*Aminocholestane hydrochloride*, crystallised in needles (from methanol–ethyl acetate), m. p. 330—334° (decomp.), $[\alpha]_D$ 24° (*c* 1·08) (Found: C, 76·1; H, 11·7%; equiv., 423. $C_{27}H_{49}N,HCl$ requires C, 76·5; H, 11·8%; equiv., 424).

The hydrochloride of 2α-dimethylaminocholestane seemed to be amorphous: the *hydrobromide* crystallised from ethyl acetate in needles, m. p. 226—228°, $[\alpha]_D$ 31° (*c* 0·72) (Found: C, 69·1; H, 10·6%; equiv., 498. $C_{29}H_{53}N,HBr$ requires C, 70·15; H, 11·0%; equiv., 497).

2β-*Aminocholestane hydrochloride*, crystallised from methanol–ethyl acetate, had m. p. 299—302° (decomp.), $[\alpha]_D$ 30° (*c* 0·94) (Found: C, 75·9; H, 11·8%; equiv., 424).

2β-Dimethylaminocholestane also formed an amorphous hydrochloride: the *hydrobromide* separated from ethyl acetate–light petroleum in needles, m. p. 253—254°, $[\alpha]_D$ 68·5° (*c* 0·89) (Found: C, 68·1; H, 10·8%; equiv., 497).

3α-Aminocholestane hydrochloride [16] had m. p. 260° (Found: equiv., 425).

3α-Dimethylaminocholestane,[17] crystallised from acetone in plates, m. p. 89·5—91°, $[\alpha]_D$ 24·5° (*c* 0·76) (Found: equiv., 414. Calc. for $C_{29}H_{53}N$: equiv., 416).

3β-Aminocholestane hydrochloride [15] had m. p. 325—328° (Found: equiv., 422).

3β-Dimethylaminocholestane,[15] crystallised from ethanol in prisms, m. p. 103—104°, $[\alpha]_D$ 23° (*c* 0·97) (Found: equiv., 415).

[15] Dodgson and Haworth, *J.*, 1952, 67.
[16] Labler, Czerny, and Šorm, *Chem. Listy*, 1954, **48**, 1058; Evans, Shoppee, and Summers, *Chem. and Ind.*, 1954, 1535.
[17] Haworth, McKenna, and Powell, *J.*, 1953, 1110.

6α-Aminocholestane hydrochloride [18] after recrystallisation from methanol–ethyl acetate had m. p. 329—333° (decomp.), $[\alpha]_D$ 34° (*c* 0·76) (Found: C, 76·5; H, 11·6%; equiv., 421).

6α-*Dimethylaminocholestane hydrochloride*, crystallised from methanol–ethyl acetate, had m. p. 251—254° (decomp.), $[\alpha]_D$ 47° (*c* 0·77) (Found: C, 76·5; H, 12·0%; equiv., 453. $C_{29}H_{53}N$,HCl requires C, 77·0; H, 12·0%; equiv., 452).

6β-*Aminocholestane hydrochloride*, crystallised from aqueous methanol in needles, m. p. 128°, $[\alpha]_D$ 4° (*c* 0·71) (Found: C, 76·4; H, 11·8%; equiv., 432).

6β-*Dimethylaminocholestane hydrochloride*, crystallised from methanol–ethyl acetate, had m. p. 237—240°, $[\alpha]_D$ 15° (*c* 1·03) (Found: C, 77·0; H, 11·8%; equiv., 454).

7α-*Aminocholestane hydrochloride*, crystallised from methanol, had m. p. 141—143°, $[\alpha]_D$ −1° (*c* 0·8) (Found: C, 76·6; H, 11·8%; equiv., 423).

7α-*Dimethylaminocholestane hydrochloride*, crystallised from methanol–ethyl acetate, had m. p. 280° (decomp.), $[\alpha]_D$ −5° (*c* 0·83) (Found: C, 77·1; H, 12·2%; equiv., 454).

7β-*Aminocholestane hydrochloride*, crystallised from methanol, had m. p. 290° (decomp.), $[\alpha]_D$ +50° (*c* 0·79) (Found: C, 76·7; H, 11·9%; equiv., 424).

7β-*Dimethylaminocholestane hydrochloride*, crystallised from ethyl acetate–light petroleum, had m. p. 211—213°, $[\alpha]_D$ 40° (*c* 0·44) (Found: C, 76·4; H, 12·1%; equiv., 454).

Cyclohexylamine hydrobromide [19] had m. p. 197·5—198·5° (Found: equiv., 184. Calc. for $C_6H_{13}N$,HBr: equiv., 180).

Dimethylcyclohexylamine hydrobromide [20] had m. p. 200—201° (Found: equiv., 210. Calc. for $C_8H_{17}N$,HBr: equiv., 208).

Dr. J. McKenna very kindly supplied the three following amines: 3α-dimethylaminocholest-5-ene, m. p. 70—71° (Found: equiv., 419. Calc. for $C_{29}H_{51}N$: equiv., 414); 3β-dimethylaminocholest-5-ene, m. p. 150—151° (Found: equiv., 418); and 3β-dimethylaminocoprostane, m. p. 62° (Found: equiv., 421).

Professor S. Šorm kindly supplied 3α-dimethylaminocoprostane, m. p. 74° (Found: equiv., 417).

Measurement of Dissociation Constants.—The amine or ammonium salt (about 15 mg.) was dissolved in equal volumes (15 ml. of each) of t-butyl alcohol (which had been distilled from sodium) and distilled water (free from carbon dioxide). While the solution was stirred under nitrogen, carbonate-free 0·05N-sodium hydroxide or 0·05N-hydrochloric acid was added dropwise from a burette that could be read to 0·005 ml. The hydroxide was standardised against potassium hydrogen phthalate. The pH values were measured with a glass electrode, a calomel electrode directly immersed, and a Doran pH-meter, calibrated with buffers at pH 3·98 and 9·20. The temperature was 23° ± 1°. The equivalent weight was determined from the end-point. The pK_a value, determined graphically from the point of half-neutralisation, was reproducible within ±0·03.

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[18] Vanghelovici and Vasiliu, *Bull. Soc. chim. Romania*, 1935, **17**, 249; *Chem. Abs.*, 1936, **30**, 7119.
[19] Osterburg and Kendall, *J. Amer. Chem. Soc.*, 1920, **42**, 2619.
[20] Wieland, Schöpf, and Hermsen, *Annalen*, 1925, **444**, 66.