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Acceleration of Hemolysis in Relation to Chemical Structure. III. The C-10 Alcohols.

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The investigation with which this note is concerned is a continuation of an inquiry into the relation between the chemical structure of various substances and the extent to which they accelerate hemolysis by simple hemolysins.[1] Through the kindness of Dr. F. C. Schmelkes I have been able to obtain purified samples of 4 C-10 alcohols, all of which, to a greater or lesser extent, are accelerators of saponin hemolysis.

Method. This has been described,* and consists in comparing time-dilution curves for the lysin (saponin) acting on washed red cells (oxalated rabbit blood) in the presence of varying quantities of the accelerator. The comparison gives a constant R; (R-1) divided by the concentration c of the accelerator in mM/l provides a satisfactory measure of the accelerating power of the substance.

The solubility of the C-10 alcohols is very small, and great care must be taken that they are really in solution. The procedure is to add a weighed quantity of the C-10 alcohol dissolved in 1 ml of ethyl alcohol to 1000 ml of water in a large glass-stoppered bottle. The bottle with its contents is vigorously shaken every hour or so for several days, first at room temperature and then when warmed to about 60°C, or until the material appears to be completely dissolved. If it is not completely dissolved, a smaller quantity is weighed out, and the process repeated. Sufficient NaCl is added finally to make the solution isotonic. The small amount of ethyl alcohol present has no accelerating or inhibiting effect on saponin hemolysis. The maximum concentration c_m obtained and used in the case of each alcohol, together with the molecular weights (M.W.) and the density (D), are given in Table I.

Saponin in dilutions of 1 in 10,000 to 1 in 60,000 in 1% NaCl was used as the lysin throughout, and the time-dilution curves were obtained at 25°C. In analyzing them, I encountered the difficulty of a diminishing R-value near the asymptotes; this complication has

[1] Ponder, E., *J. Exp. Biol.*, 1939, **16**, 38, acceleration by benzene derivatives; Ponder, E., and Hyman, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 320, acceleration by straight-chain alcohols.

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TABLE I.
Accelerating Power of C-10 Alcohols for Saponin Hemolysis.

	M.W.	D.	c_m mM/l	(R—1)/c	× benzene (0.025 mM/l)
Nonyl alcohol	144	0.84	0.5	—0.8	32
n-decanol	158	0.83	0.2	—2.1	83
Tetrahydro-geraniol	158	0.86	0.2	—1.0	40
Citronellol	156	0.88	0.2	—0.6	24
Geraniol	154	0.85	0.2	—0.1	4

been fully discussed elsewhere,[2] and the R-values given in Table I are those obtained from parts of the curves well below the asymptotes, *i. e.*, where R is relatively constant. In the last column of the table, each accelerator is compared with benzene as regards potency, benzene having been used as a kind of reference substance in work of this kind.

Results. These are shown in Table I, and lead to 2 conclusions. 1. The accelerating power of the straight-chain C-10 alcohol, n-decanol, is considerably less than that calculated from the expression given by Ponder and Hyman as relating the accelerating power $A = (1\text{-}R)/c$, to the number of carbon atoms N. The expression, $N = 1.75 \cdot \log A + 9$, gives 9.6 for N instead of 10, or, to have $N = 10$, A would have to be 3.7 instead of 2.1; the observed accelerating power is accordingly only about half of that predicted. 2. Among the C-10 alcohols themselves, small differences in structure correspond to considerable differences in accelerating power. Thus tetrahydro-geraniol, a branched chain alcohol with the same molecular weight as n-decanol, produces only about half the acceleration of the latter, whereas the introduction of one double bond, as in citronellol, reduces the accelerating power still further, and the presence of two double bonds, in geraniol, almost abolishes it. In most instances where comparisons of this kind have been made, the differences in accelerating power have corresponded to greater differences in molecular structure (the length of the carbon chain, the number of added halogens, etc.) than we have here.

Conclusion. The accelerating power of 4 C-10 alcohols on saponin hemolysis is in the order of n-decanol>tetrahydro-geraniol>citronellol>geraniol, these differences corresponding to small structural differences in the accelerating molecule.

[2] Ponder, E., *J. Gen. Physiol.*, 1941, **25**, 247.