with toluene for a few days at 0° until the electrophoresis measurements could be completed.

In our experiments we have employed the electrophoresis cell described by Northrop,[6] making measurements under the microscope of the migration velocity of the particle or an aggregate and reversing the applied potential repeatedly. The measurements at a given *p*H and recorded in Table I, are the mean of ten or twelve closely agreeing observations taken both at the upper and lower stationary levels for the cell. The data are shown graphically in Fig. 1.

TABLE I

ELECTROPHORETIC VELOCITY AND CALCULATED ζ-POTENTIAL OF ASCLEPAIN

*p*H	Velocity μ/sec./v/cm.	Calcd. ζ-potential, millivolts
1.82	+2.03	+25.6
2.15	+1.58	+19.9
2.50	+1.01	+12.7
2.69	+0.74	+ 9.3
2.90	+ .38	+ 4.8
3.00	+ .18	+ 2.2
3.23	− .21	− 2.6
3.45	− .59	− 7.4
3.60	− .90	−11.3
3.86	−1.31	−16.5
4.05	−1.58	−19.9
4.30	−1.85	−23.3

(6) Northrop, *J. Gen. Physiol.*, **4**, 629 (1922).

From the Helmholtz-Lamb equation $V = \zeta HD/4\pi h$, where V is the velocity of particle, ζ is electrokinetic potential, H is potential gradient per cm., D is dielectric constant of the dispersion medium and h is viscosity of the medium. The values of D and h have been assumed to be those for water, namely, 81 and 0.009, respectively, at 25°. All quantities in the above equation are expressed in c. g. s. electrostatic units.

Not until our observations were completed did we note the statement by Winnick, Davis and Greenberg (ref. 3, p. 277) that from the insolubility of their asclepain at *p*H 3.2 they expected the isoelectric point would lie in this region. That our preparation is the same material employed by Greenberg and co-workers is evident from our value of *p*H 3.11 for the isoelectric point and from the similar proteolytic properties observed in both laboratories.

Summary

1. The electrophoretic velocity of asclepain has been measured in citrate, acetate and phosphate buffer solutions in the *p*H range 1.8 to 8.0 and the electrical charge on the particle calculated.

2. The isoelectric point of asclepain was found to be at *p*H 3.11 or C_H 7.8 × 10^{-4}.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SCHIEFFELIN & CO.]

Mercurials from Aliphatic Glycols

BY ANTHONY J. SHUKIS AND RALPH C. TALLMAN

The relationship between the distribution coefficients of compounds which can be distributed between water and a lipoid or a lipoid solvent and their anti-bacterial activity has attracted considerable attention.[1] It appears to be rather well established that in a given series of compounds with differing distribution coefficients those which are distributed in favor of the lipoid or the lipoid solvent are the better anti-bacterial agents.[1] In connection with more extensive synthetic work, three series of mercury-containing organic compounds were prepared which afforded the opportunity to test again the reliability of this general concept.

All the syntheses were performed by the well-known reaction[2] whereby an alcoholic hydroxyl group and mercuric compounds such as mercuric acetate add, in effect, to a molecule containing an ethylenic double bond

$$R{-}OH + (AcO)_2Hg + CH_2{=}CH_2 \longrightarrow R{-}O{-}CH_2{-}CH_2{-}Hg{-}OAc + AcOH$$

(1) Harden and Reid, THIS JOURNAL, **54**, 4325 (1932); Dunning, Dunning and Reid, *ibid.*, **55**, 1565 (1936); Hurd and Fowler, *ibid.*, **61**, 249 (1939), and others.

(2) Wright, *ibid.*, **57**, 1994 (1935), and others.

In this work, the acetate group in the final product was converted to the chloride. In general the alcohols used were polyethylene glycols, the monoethyl ethers of polyethylene glycols, or polymethylene glycols. The unsaturated compound in every instance was ethylene. Compounds of the following types were produced for the most part

I $CH_3{-}CH_2{-}(O{-}CH_2{-}CH_2)_n{-}O{-}CH_2{-}CH_2{-}Hg{-}Cl$

II $HO{-}CH_2{-}CH_2{-}(O{-}CH_2CH_2)_n{-}O{-}CH_2{-}CH_2{-}Hg{-}Cl$

III $HO{-}(CH_2)_n{-}O{-}CH_2{-}CH_2{-}Hg{-}Cl$

In addition to these general types, several specific derivatives of them were prepared for purposes of comparison. The physical properties of all compounds are included in the table.

It was possible to alter the distribution coefficients of the compounds within the scope of these series by changing the ratio of —OH and —O— to the —CH_2— groups within a molecule. With all compounds except two (see table) monomercuration took place, even where more than one —OH group was present in the starting material. In these instances, with tri- and tetraethylene glycols as starting materials, dimercurials were

obtained. Various modifications of the experimental procedure were resorted to in attempting to obtain monomercurials from these glycols but without success. Conversely, we were unable to prepare dimercurials of the mono and diethylene glycols. Apparently, insofar as this series has been carried, ethylene glycol and diethylene glycol yield monomercurials by the method used, while higher members of the series dimercurate. No adequate explanation for this behavior has suggested itself.

The mercurials were distributed between benzene and water and the ratio of their solubilities in each solvent, K_D, solubility in benzene/solubility in water, was obtained.

The correlation between the distribution coefficients and anti-bacterial activity finds good agreement with previously reported data for other types of compounds (see table). In our work, in a given series of compounds the distribution coefficients, K_D(solubility in benzene/solubility in water) are directly proportional to the anti-bacterial potency of a compound. The greater K_D, the better is the compound as an anti-bacterial agent. All three groups, as a whole, show good agreement with the generalization. Special attention is called to compounds 9, 10 and 11, 12 and 13. In each of these two groups, the carbon skeleton is the same, but substituents altering the distribution coefficients have been introduced with the consequent expected effect on the anti-bacterial potency.

Bacteriostatic activity against *Staph. aureus* of each compound is shown in column 6 of the table. Further details of the bacteriological examination will appear elsewhere.

TABLE I

FORMULAS, PHYSICAL CONSTANTS, ANALYSES

No.	n	M. p., °C.	Anal., % Hg Calcd.	Found	K_D	Bacteriostatic dilution $\times 10^{-6}$
Type I, CH_3-CH_2-(O-CH_2-CH_2)$_n$-O-CH_2-CH_2-Hg-Cl						
1	0[a]	92	64.8	64.0	14.0	2.5
2	1[b]	34–35	56.8	55.2	7.8	1.3
3	2[b]	50	50.5	50.1	7.3	1.0
4	3	53–54	45.5	45.6	3.9	0.5
5	4	oil	41.4	40.9	2.1	0.16
Type II, HO-CH_2-CH_2(O-CH_2-CH_2)$_n$-O-CH_2-CH_2-Hg-Cl						
6	1	70–72	54.5	53.0	3.6	0.6
7	2[c]	90–91	58.7	59.5	48.0	0.4
8	3[c]	86–87	55.7	55.7	15.2	0.3
Type III, HO-(CH_2)$_n$-O-CH_2-CH_2-Hg-Cl						
9	2	88–89	61.7	60.0	0.06	0.8
10	2[d]	54	58.4	59.5	29.3	2.5
11	3	114–116	59.2	60.6	6.0	1.3
12	[e]	80–81	59.2	59.3	3.8	1.4
13	[f]	88–91	56.5	56.6	0	0.5
14	4	92–93	56.8	55.5	0.46	1.4
15	6	98–99	52.6	52.2	3.7	2.5

[a] *Cf.* Schoeller, *et al.*, *Ber.*, **46**, 2864 (1913). [b] *Cf.* Ellingsworth, *et al.*, British Patent 469,022 in which compounds were mentioned as oils with no analyses given. [c] Dimercurials with -HgCl on both ends. [d] Prepared from ethylene chlorohydrin; differs from type formula by Cl in place of -OH. [e] Compound prepared from propylene 1,2-glycol and is isomer of 11 although exact structure has not been determined. [f] Compound prepared from glycerol and has one more -OH than type formula although exact structure has not been determined. Insoluble in benzene.

Experimental

Starting Materials.—The starting materials for compounds of Type I are, in addition to ethyl alcohol for the simplest member of the series, the monoethyl ethers of polyethylene glycols. These were either purchased or prepared by the method of Cretcher and Pittenger.[3] Polyethylene glycols for Type II compounds were purchased as such and redistilled before use. Polymethylene glycols, the starting materials for Type III compounds, were purchased or prepared by hydrogenating the esters of corresponding dicarboxylic acids.

Mercuration of Glycols.—The method of preparation of the mercury-containing compounds was similar in each instance. In general, 0.1 mole of mercuric acetate was suspended in 1.0 mole of the alcohol or glycol contained in a 500-ml. three-necked flask equipped with a mechanical stirrer, a calcium chloride tube and a gas inlet tube. The flask was maintained at 70–90° by means of a water-bath while ethylene gas from a cylinder was admitted at such a rate that the bubbles could just be counted while the suspension was stirred thoroughly. At the end of one-half to one hour, the suspended mercuric acetate had dissolved and a slight turbidity remained. Completeness of the reaction was determined by testing for mercuric ion in solution. (In the presence of mercuric ions, addition of alkali to a sample gives a yellow precipitate of HgO; while the addition of iodide ion to a sample gives a red precipitate of HgI_2 soluble in excess iodide ion.) When the reaction was found to be complete, the mixture was filtered, the filtrate distilled to remove the excess of solvent if the latter was not water soluble, and the residue poured into an equal volume of 20% aqueous sodium chloride solution. If the solvent is water soluble, the mixture may be poured directly into the salt solution without distillation. The white precipitate of the chloromercurial was filtered and the compound recrystallized from water or water and ethyl alcohol. The yields varied from 20–60%. The mercury content of the compounds was determined by the Rauscher method as modified by us.[4]

Distribution Coefficients.—For distribution studies, 0.2–0.3 g. of the mercurial was weighed accurately and dissolved in 100 ml. of benzene in a volumetric flask. Two 10-ml. aliquots were removed and the mercury content determined,[4] giving (A). Of the solution that remained, 75 ml. was measured out and placed in a shaking bottle. An equal volume of distilled water was added and the mixture mechanically shaken for two hours. After standing for an additional hour to allow complete separation of the layers, two 10-ml. aliquots were removed from the benzene layer and analyzed for mercury, giving (B). K_D was calculated as B/A-B. The distribution was repeated in each case and the average values recorded.

Summary

The preparation of three series of alkoxy mercurials is reported. Their distribution between water and benzene has been determined, and an attempt made to correlate the anti-bacterial activity of these compounds with their distribution coefficients.

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(3) Cretcher and Pittenger, THIS JOURNAL, **46**, 1503 (1924).

(4) Shukis and Tallman, *Ind. Eng. Chem., Anal. Ed.*, **12**, 123 (1940).