Paper Chromatography of Steroid Glucuronosides and Sulphates

We wish to report the separation by paper chromatography of a number of steroid glucuronosides and sulphates. The composition of the three systems reported and the conjugates used are given in Table 1, together with the R_F values noted in a representative experiment. 10–50 gamma amounts of the free acids or their sodium salts in methanol were spotted on 22½-in. lengths of Whatman No. 1 paper and, after equilibration overnight at room temperature in paper-lined glass tanks, the mobile phase was added to the trough and descending chromatography was carried out for periods of 4–8 hr. Following chromatography, the papers were dried in a current of air at room temperature, and the conjugates were detected by spraying with, or dipping in, 2–4 per cent phosphomolybdic acid in absolute ethanol followed by heating at 90°–100° C. for 5 min. Conjugate 2 was also detected by dipping in 15 per cent aqueous phosphoric acid, followed by heating at 90° C. for 20 min. and examination under ultra-violet light[1], or by dipping in a triphenyltetrazolium chloride reagent prepared by combining immediately before use one volume of 2 per cent triphenyltetrazolium chloride in water with four volumes of 10 per cent sodium hydroxide in 60 per cent aqueous methanol.

In these experiments the ambient temperature varied from 23° to 33° C.; but in the course of a single experiment the temperature change usually did not exceed 3 degrees. Although R_F values varied slightly with temperature, it was possible to use these systems at temperatures as low as 4° C. and as high as 38° C.

System I has been used chiefly to separate conjugates 1 and 2 recovered from urine following the administration of tetrahydrocortisone (pregnane-3α, 17α, 21-triol-11,20-dione)[2]. Substituting formic acid for acetic acid, as in system II, specifically increases the R_F values of the sulphates and diminishes tailing. This system is the most useful one so far developed for separating the three sulphates. System III separates the several glucuronosides from one another and from the sulphates, but does not separate completely the three members of the sulphate group. Fig. 1 illustrates the resolution of a mixture of conjugates 1, 2, 3, 4, 5 and 7 using system III. All three systems separate effectively conjugate 3 from conjugate 5 and, presumably, would separate other sulphates and glucuronosides having a common steroid moiety.

Table 1. R_F Values of Steroid Conjugates Chromatographed on Paper

Experiment 22*A*. Equilibration time, 14½ hr. at 29·7°–32·0° C. Running time, 5½ hr. at 28·5°–29·7° C.

Conjugate		Chromatographic system		
No.	Identity	I	II	III
1	β-Cortolone monoglucuronoside (crude)	0·14	0·16	0·08
2	Tetrahydrocortisone monoglucuronoside	0·39	0·42	0·13
3	Dehydro*iso*androsterone glucuronoside	0·78	0·78	0·21
4	Pregnanediol monoglucuronoside	0·81	0·82	0·27
5	Sodium dehydro*iso*androsterone sulphate	0·25	0·34	0·42
6	Sodium androsterone sulphate	0·38	0·47	0·45
7	Sodium pregnenolone sulphate	0·40	0·52	0·52

System I: *n*-Butyl acetate – *n*-butanol – 10 per cent acetic acid (80 : 20 : 100).
System II: *n*-Butyl acetate – *n*-butanol – 10 per cent formic acid (80 : 20 : 100).
System III: *n*-Butyl acetate – methanol – 0·1 *M* sodium barbital buffer in 50 per cent aqueous methanol, *p*H 8·2 (150 : 50 : 50) (monophasic).

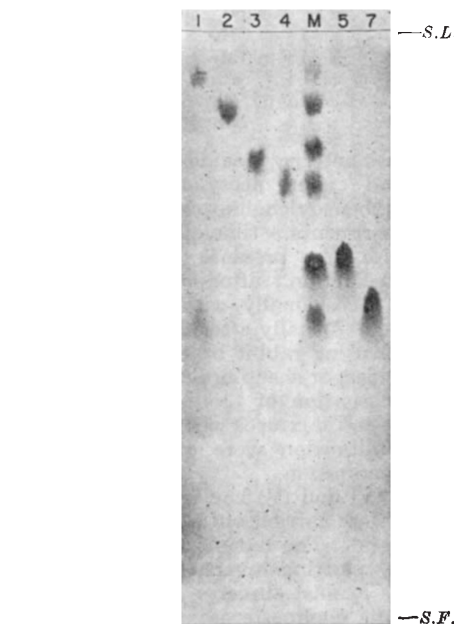

Fig. 1. Resolution of a mixture (*M*, above) of conjugates 1, 2, 3, 4, 5 and 7 using system III. Running time, 5½ hr. at 30·5°–32·5° C. *S.L.* designates starting line and *S.F.* solvent front

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[1] Neher, R., and Wettstein, A., *Helv. Chim. Acta*, **34**, 2278 (1951).
[2] Schneider, J. J., Lewbart, M. L., Levitan, P., and Lieberman, S., *J. Amer. Chem. Soc.*, **77**, 4184 (1955).

Inhibition of Cholinesterases by 1 : 2 : 4-Triazoles

It has been repeatedly observed in my laboratory that workers, including non-smokers, manipulating 1 : 2 : 4-triazoles develop symptoms of light nicotine or physostigmine poisoning from time to time. Subsequent experiments have shown that some simple water-soluble 1 : 2 : 4-triazoles may act as inhibitors of cholinesterase activity.

Fresh brains of sheep or rabbit homogenized in 0·025 *M* sodium bicarbonate were used as sources of cholinesterase. Sheep brains were satisfactory, but experiments with rabbit brains could not be satisfactorily duplicated. The inhibitors tested were 3 : 5-dimethyl (I)-, 1 : 3 : 5-trimethyl (II)-, 3 : 5-dimethyl-1-phenyl (III)-, 3-ethyl-5-methyl-1-phenyl (IV)- and 5-ethyl-3-methyl-1-phenyl (V)-1 : 2 : 4-triazoles.

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