information which allowed D for the solute to be found. While this procedure cancels out any variation in the properties of the filter papers used as diaphragms, it makes no allowance for the enhancement or retardation of solute flux caused by interaction with the flux of the standard. We believe that the reproducibility of our results, in particular those obtained for the calibration of the cell, indicate that the Millipore discs employed are of an acceptably consistent standard. Having calibrated the cell for a particular solvent, therefore, the straightforward technique and analysis described in this paper provides a fast, reliable and reproducible means for diffusion coefficient determination.

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REFERENCES

Albery, W. J., Burke, J. F., Leffler, E. B., Hadgraft, J. (1976) J. Chem. Soc. Faraday Trans, 1 72: 1618–1626

Albery, W. J., Hadgraft, J. (1979) J. Pharm. Pharmacol. 31: 65–68

Armstrong, N. A., James, K. C., Wong, K. C. (1979) Ibid. 31: 627–631

Blasinski, H , Boss, J , Tosick, B. (1968) Chem. Stosow Ser. B. 5: 469

Gage, J. C. (1948) Trans. Faraday Soc. 44: 253–256

Gosting, L. J. (1950) J. Am. Chem. Soc. 72: 4418–4422

Harned, H. S., Nuttall, R. L. (1947) Ibid. 69: 736–740

Harned, H. S., Nuttall, R. L. (1949) Ibid. 71: 1460–1463

Kreevoy, M. M., Wewerka, E. M. (1967) J. Phys. Chem. 71: 4150–4152

Poulsen, B. J., Young, E., Coquilla, V., Katz, M. (1968) J. Pharm. Sci. 57: 928–933

Robinson, R. A., Stokes, R. H. (1965) Electrolyte Solutions. 2nd ed. (rev.) Butterworths, London, pp 253–261

Stokes, R. H. (1950a) J. Am. Chem. Soc. 72: 763–767

Stokes, R. H. (1950b) Ibid. 72: 2243–2247

Stokes, R. H. (1951) Ibid. 73: 3527–3528

Tuwiner, S. B. (1962) Diffusion and Membrane Technology. Reinhold Publishing Corp., New York, pp 369–373

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Isolation and identification of probenecid acyl glucuronide

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Purification of isolated and synthesized glucuronides has often proved difficult and has frequently resulted in impure gums. We have isolated probenecid acyl glucuronide (PAG), a glucuronide not previously isolated in the pure form or fully characterized (Perel et al 1971), using reversed phase high performance liquid chromatography and ^{13}C nuclear magnetic resonance. Separations were performed with a Waters Associates ALC/GPC 244 liquid chromatograph with a μBondapak-C_{18} column, and methanol–water–acetic acid (40:59:1) as solvent. ^{13}C n.m.r. spectra were obtained using a Varian F T–80A instrument, with DMSO-d_6 as solvent.

A single 2·0 g oral dose of probenecid was administered to a volunteer and urine was collected for 52 h. A sample of each voiding was filtered through a 0·45 μm membrane filter and 10 μl analysed by h.p.l.c. to assess the quantity of probenecid metabolites present. Fig. 1 shows the results for a voiding after 6·5 h. The metabolites (excluding PAG) were synthesized (Guarino et al 1969; Conway & Melethil 1974) and peak A was shown to be *p*-(propylsulphamoyl) benzoic acid, while peak B was a mixture of *p*-(*N*-propyl *N*-2-hydroxypropylsulphamoyl) benzoic acid and *p*-(*N*-propyl N-2-carboxyethylsulphamoyl) benzoic acid. (A μBondapak-CN column using as solvent methanol–water–acetic acid (35:63:1) resolved all the known metabolites but this column did not have sufficient resolution for preparative loading and collection of the group of compounds labelled C and peak D).

Peaks C and D were collected and evaporated. Hydrolysis of either (5M HCl, reflux 1 h) yielded probenecid (identified by h.p.l.c.) whereas reduction of either using diborane yielded glucose (p.c. and g.l.c.).

A urine sample (250 ml) was acidified (25 ml of 5M HCl) and extracted with ethyl acetate (3 × 250 ml). After evaporation, the organic phase was partitioned between chloroform and water (250 ml of each). The aqueous phase was extracted with ethyl acetate (3 × 250 ml) and the organic layer evaporated. The residue was taken up in methanol (1 ml) and 200 μl injected into the h.p.l.c. Peaks C and D were collected and the mobile phase evaporated. After reinjection of the collected fractions, using as solvent methanol–aqueous phosphoric acid pH 3 (40:60), C was shown to be a mixture of at least two compounds whereas fraction D was a single compound. This solvent system gave better resolution than the acetic acid system but acetic acid was preferred for preparative chromatography as it is easily removed from collected fractions.

Fraction D was amorphous to X-rays and had m.p. 93–95 °C and optical rotation $[\alpha]_D^{20}$ −4·1 (c = 0·32 in methanol). Its elemental composition of $C_{19}H_{27}NO_{10}S$ (found: C, 49·05; H, 6·11; N, 2·94; S, 7·38 and calc: C, 49·45; H, 5·90; N, 3·04; S, 6·95%) is consistent with the structure of D being a glucuronic acid conjugate of

* Correspondence.

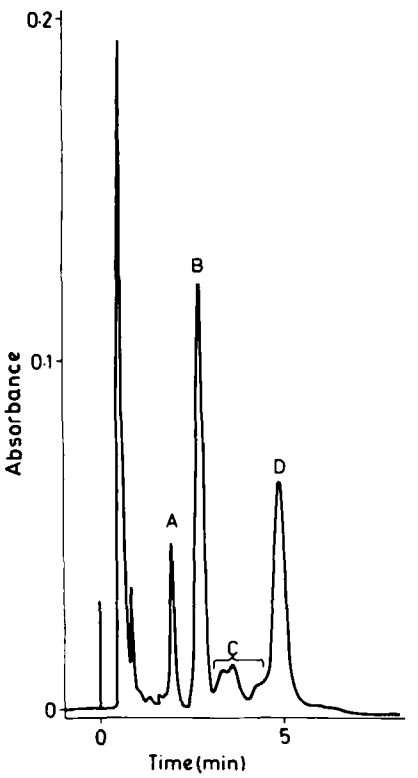

FIG. 1. Analytical h.p.l.c. trace of human urine 6·5 h after ingestion of a 2·0 g oral dose of probenecid. Injection volume: 10 μl. Column: μBondapak C_{18}. Solvent: methanol–water–acetic acid (40:59:1).

probenecid. The ^{13}C n.m.r. spectrum [(δ ppm, multiplicity and coupling constants in proton coupled spectrum, carbon assignment): 169·8, singlet, COOH; 163·6 singlet, COOR; 144·3, triplet J = 8·0 Hz, >C=; 132·3, triplet J = 7·7 Hz, >C=; 130·7, doublet of doublets J = 166·0, 5·7 Hz, =CH–; 127·3, doublet of doublets J = 169·0, 5·5 Hz, =CH–; 95·4, doublet J = 166·1 Hz, O–CH–O; 76·3, doublet J = 149·3 Hz, >CH–O; 75·6, doublet J = 144·3 Hz, >CH–O; 72·3, doublet J = 143·6 Hz, >CH–O; 71·4, doublet J = 146·4 Hz, >CH–O; 49·6, triplet J = 136 Hz, $-CH_2-N$; 21·5, triplet J = 129 Hz, $>CH_2$; 11·0, quartet J = 126, CH_3)] displays fourteen signals of which those arising from probenecid are easily identified (8 signals).

The anomeric carbon signal for PAG is found at 95·4 ppm; for α-D-glucuronic acid it occurs at 93·2 ppm and for the β-form 96·9 ppm (Pfeffer et al 1979). Acylation of C_1 of glucose causes a upfield shift (Breitmaier et al 1975) and so the glucuronic acid is linked through C_1 to probenecid and is, as expected, in the β configuration. The C_6 carboxyl signal is at 169·8 ppm and the carboxyl linked to C_1 is assigned to the signal at 163·6 ppm due to the upfield shift resulting from esterification (Stothers 1972) with probenecid. Hence fraction D is PAG.

Fraction C was shown to be a mixture of those compounds in which the probenecid moiety is attached to C_2, C_3 and C_4 of glucuronic acid (Bray 1953). These compounds were prepared by treating PAG with 50mM aqueous ammonia for 1 min and some had the same retention times as fraction C. The ^{13}C n.m.r. spectrum of C showed signals at 92·9 and 97·1 ppm confirming that both anomers were present and that the linkage was not through C_1. This migration of the aglycone did not occur on the h.p.l.c. column as injection of PAG gave rise to only one peak. Each urine sample was analysed by direct injection of 10μl of urine on to the h.p.l.c. column (Fig. 1) as soon as possible after voiding and did not involve any sample preparation. Thus the compounds are either genuine metabolites or they are products of rearrangement of PAG subsequent to glomerular filtration.

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REFERENCES

Bray, H. G. (1953) in: Hudson, C. S., Wolfrom, M. L. (eds) Advances in Carbohydrate Chemistry. Volume 8 Academic Press, New York, pp 254–256

Breitmaier, E., Haas, G., Voelter, W. (1975) Atlas of Carbon-13 NMR Data. Heydon, London, pp 902–909

Conway, W. D., Melethil, S. K. (1974) J. Pharm. Sci. 63: 1551–1554

Guarino, A. M., Conway, W. D., Fales, H. M. (1969) Eur. J. Pharmacol. 8: 244–252

Perel, J. M., Dayton, P. G., Yu, T. F., Gutman, A. B. (1971) Eur. J. Clin. Pharmacol. 3: 106–112

Pfeffer, P. E., Valentine, K. M., Parrish, F. W. (1979) J. Am. Chem. Soc. 101: 1265–1274

Stothers, J. B. (1972) Applied Spectrosc. 26: 1–16