CHROM. 5062

Gas chromatographic assay of phenylbutazone in biological fluids

The quantitative estimation of phenylbutazone (I) in biological fluids is very difficult since only a small amount of the drug is present. The presence of its metabolites [*i.e.*, 1-phenyl-2-*p*-hydroxyphenyl-3,5-dioxo-4-*n*-butylpyrazolidine (II) and 1,2-diphenyl-3,5- dioxo-4-(3-hydroxybutil)-pyrazolidine (III)][1,2], as well as other matter that interferes with the assay, complicates the quantitative estimation. The various methods consisting of selective extractions of I followed by a spectrophotometric determination[2–5] are clearly unsatisfactory for the specificity.

Our attempts to overcome the drawbacks mentioned above by using paper or thin-layer chromatography were unsatisfactory, due to some decomposition observed for I just after it was applied. This phenomenon is quite similar to that reported by MACEK[6] for a number of substances of the pyrazolone type. While seeking an alternate method which would be more sensitive and specific, we turned our attention to achieving by gas chromatography the quantitation of I in biological fluids. Promazine was chosen as an internal standard because its retention time was found to be very close to that of I (15 and 17.5 min, respectively, under our working conditions).

Experimental

Equipment and working conditions. A Pye 25 Series 104 gas chromatograph fitted with a dual flame ionisation detector head and coiled Pyrex glass columns (150 × 0.4 cm) was used. The columns were packed with 2.5% silicone rubber SE-30 on 100–120 mesh Gas-Chrom Q. The working conditions were: column temperature, 195°; nitrogen flow, 40 ml/min; hydrogen flow, 40 ml/min; air flow, 400 ml/min.

Assay. A mixture of 1–200 μg of I, 10 μg–2 mg of II and 10 μg–2 mg of III was dissolved in 0.1 ml of acetone and was then added to 1 ml of rat serum or urine. The sample was then acidified with 2 ml of 1 *N* HCl and extracted by shaking for 30 min with 20 ml of highly pure *n*-heptane. The organic phase was separated, dried over

TABLE I

RECOVERY OF PHENYLBUTAZONE FROM RAT SERUM

Sample	*Phenylbutazone added (μg)*	*1-Phenyl-2-p-hydroxyphenyl-3,5-dioxo-4-n-butyl-pyrazolidine added (μg)*	*1,2-Diphenyl-3,5-dioxo-4-(3-hydroxybutyl)-pyrazolidine added (μg)*	*Phenylbutazone found (μg)*	*Δ %*
1	1.25	11.98	10.75	1.31	+4.80
2	1.13	11.76	10.51	1.18	+4.43
3	50.85	500.71	499.31	49.65	−2.36
4	50.05	501.07	502.25	50.50	+0.90
5	101.10	1002.05	998.50	102.50	+1.39
6	99.68	999.57	1000.35	100.52	+0.84
7	149.95	1495.75	1501.57	148.00	−1.30
8	150.38	1502.37	1498.35	154.15	+2.51
9	201.28	2002.25	1997.75	203.75	−1.23
10	200.15	2001.50	2003.24	197.50	−1.32

TABLE II

RECOVERY OF PHENYLBUTAZONE FROM RAT URINE

Sample	*Phenylbutazone added* (μg)	*1-Phenyl-2-p-hydroxyphenyl-3,5-dioxo-4-n-butyl-pyrazolidine added* (μg)	*1,2-Diphenyl-3,5-dioxo-4-(3-hydroxybutyl)-pyrazolidine added* (μg)	*Phenylbutazone found* (μg)	Δ%
1	1.15	10.85	10.58	1.21	+5.22
2	1.23	11.50	10.75	1.29	+4.88
3	51.25	505.30	500.60	53.15	+3.70
4	50.37	498.50	501.51	52.00	+3.13
5	99.81	1001.00	1005.77	95.60	−4.22
6	100.75	999.75	995.60	99.75	−0.99
7	151.37	1510.55	1500.43	154.15	−1.84
8	150.55	1505.00	1502.37	152.20	−1.10
9	200.00	2005.57	2008.17	196.50	+1.75
10	198.75	1995.68	1999.46	197.30	−0.73

Na_2SO_4, and filtered; 15 ml of this extract were then evaporated to dryness, taking care that the temperature did not exceed 20°. The residue was taken up in 1 ml of standard solution (1–200 μg of promazine dissolved in 1 ml of carbon disulfide in a ground-glass stoppered tube) and the new solution was evaporated to dryness at room temperature in a drier connected to a vacuum pump. The new residue was dissolved in 50–100 μl of carbon disulfide and 3–6 μl of this solution were injected into the gas chromatograph.

Results and discussion

Tables I and II report, respectively, the results of the gas chromatographic assay of I in rat serum and urine after addition of a mixture of I, II and III. It can be seen that the standard error did not exceed 5%. The sensitivity of this technique was 1 μg of I per ml of serum or urine. The other substances which are normally extracted from these fluids with *n*-heptane did not interfere with the assay. The metabolites of I were somewhat extracted from *n*-heptane, but they were not eluted from the gas chromatographic columns under our working conditions.

Research Laboratories, R. PEREGO
Istituto De Angeli, E. MARTINELLI
Via Serio, 15-I 20139 *Milan* (*Italy*) P. C. VANONI

1 B. B. BRODIE, J. J. BURNS, E. W. LOWMAN, T. CHENKIN, P. LEE, M. WEINER AND J. M. STEELE, *Am. J. Med.*, 16 (1954) 181.
2 J. J. BURNS, R. K. ROSE, T. CHENKIN, A. GOLDMAN, A. SCHULERT AND B. B. BRODIE, *J. Pharm. Exp. Therap.*, 109 (1953) 346.
3 R. PULVER, *Schweiz. Med. Wochschr.*, 80 (1950) 308.
4 B. HERRMANN, *Med. Exp.*, 1 (1960) 170.
5 J. E. WALLACE, *J. Pharm. Sci.*, 57 (1968) 2053.
6 K. MACEK, *J. Chromatog.*, 33 (1968) 332.

First received April 27th, 1970; revised manuscript received September 24th, 1970