Acetylation of Sulfisoxazole by Isolated Perfused Rat Kidney

Keyphrases □ Sulfisoxazole—renal metabolism to N^4-acetyl metabolite using the isolated perfused rat kidney □ Metabolism, renal—sulfisoxazole, acetylation by the isolated perfused rat kidney

To the Editor:

Although the kidneys contain many metabolizing enzymes of the liver (1), renal drug metabolism has received limited attention, due partially to the relegation of the kidneys to organs of excretion. The isolated perfused kidney is a useful technique for studies of the renal disposition of drugs. In many cases, experiments with the isolated perfused kidney give results that are consistent with data obtained from *in vivo* studies and more clearly define the role of the kidneys in the overall disposition of a drug. In our laboratory, the isolated perfused kidney has been used in renal clearance studies of several compounds (2, 3) as well as in studies of renal metabolism and interconversion of salicylic and salicyluric acids (4).

Since sulfisoxazole (I) is eliminated largely by renal excretion, it can be influenced by protein binding, urinary pH, and the urine flow rate (5, 6). Studies in our laboratory on the renal clearance of sulfisoxazole by the isolated perfused kidney indicated that rat kidneys metabolize this drug to its N^4-acetyl metabolite (II).

Male Sprague–Dawley rats, 350–375 g, were used in the isolated perfused kidney experiments. The surgical technique and experimental details were reported previously (2–4). Sulfisoxazole (6.5 mg) was administered to attain an initial concentration of ~100 μg/ml, and perfusion was continued for 90 min. The experimental time was divided into nine 10-min urine collection periods. Urine samples were assayed for sulfisoxazole and N^4-acetylsulfisoxazole by a modified high-performance liquid chromatographic assay (7).

The mean urinary excretion rates for N^4-acetylsulfisoxazole and sulfisoxazole from five perfusion experiments are presented in Table I. Although the amounts of N^4-acetylsulfisoxazole excreted in the urine were low, they accounted for 5–7% of the total drug excreted as sulfisoxazole plus N^4-acetylsulfisoxazole during the 90-min experiment. Previous *in vivo* experiments on elimination of sulfisoxazole in the rat indicated that, of the total drug excreted in the urine, 12% is excreted as the N-acetyl metabolite (8). The present results thus indicate that half of the excreted N-acetyl metabolite is due to renal metabolism and that, in the rat, the kidneys contribute significantly to the metabolic disposition of sulfisoxazole.

Table I—Urinary Excretion Rates of N^4-Acetylsulfisoxazole (II) Formed by the Perfused Rat Kidney following Administration of 6.5 mg of Sulfisoxazole (I)

Minutes	Urinary Excretion Rate, μg/10 min	
	II	I
10	0.87 ± 0.25[a]	11.12 ± 4.24[a]
20	1.16 ± 0.62	18.78 ± 10.02
30	1.08 ± 0.57	18.40 ± 5.72
40	1.74 ± 0.55	27.98 ± 12.82
50	1.58 ± 0.48	31.13 ± 18.28
60	1.45 ± 0.47	34.70 ± 18.21
70	1.58 ± 0.38	28.30 ± 8.42
80	1.53 ± 0.19	27.30 ± 6.09
90	1.43 ± 0.27	28.38 ± 7.08
Total	12.06	226.02

[a] Mean ± *SD*, $n = 5$.

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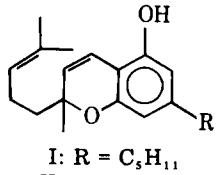
Anti-Inflammatory Activity of Cannabichromene Homologs

Keyphrases □ Anti-inflammatory activity—evaluation of cannabichromene homologs □ Cannabichromene homologs—evaluation for anti-inflammatory activity

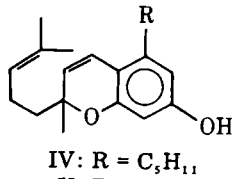
To the Editor:

As part of our continuing study of the cannabinoids, we recently reported that cannabichromene has anti-inflammatory activity. The two tests used to show this activity were the carrageenan-induced rat paw edema test and the red cell stabilization assay (1). To determine the effect of changing the length and position of the side chain of cannabichromene on its anti-inflammatory activity, four homologs were prepared and tested.

Cannabichromene-C_5 (I), cannabichromene-C_1 (II), and cannabichromene-C_0 (III) were prepared following the procedure outlined previously (2), starting from olivetol, orcinol, and resorcinol, respectively. The yield of I was



I: R = C_5H_{11}
II: R = CH_3
III: R = H



IV: R = C_5H_{11}
V: R = H

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Table I—Effectiveness of Cannabichromene and Its C_1 Homolog in Inhibiting Carrageenan-Induced Rat Paw Edema [a]

Compound	Dose, mg/kg	Inhibition, %	Mean Difference[b]	SEM	n	p[c]
Vehicle control	—	—	0.340	0.560	24	—
I	120	70	0.102	0.049	8	0.01
	240	86	0.048	0.020	8	0.001
	480	100	0.002	0.058	8	0.001
II	60	53	0.160	0.037	8	0.01
	120	63	0.126	0.031	8	0.001
	240	92	0.027	0.038	7	0.001
Phenylbutazone	60	52	0.163	0.023	8	0.01
	120	76	0.082	0.046	8	0.01

[a] Rats received intraperitoneal injections of test compounds 30 min prior to injection of carrageenan, and postinjection measurements were taken after 3 hr. [b] Preinjection volume − postinjection volume. [c] Each test group was compared to the vehicle control group using the Student t test.

~60% (molar conversion) and that of III was 48.3%, while the yield of II was only 10.8%. Although only trace amounts of the *iso*-compounds were formed from the C_5 and C_1 homologs, almost equal quantities of II and isocannabichromene-C_0 (V) were formed under the same conditions. Thus, the same reaction was used to prepare II and V. Similar yields of II and V were formed when the same reaction was carried out in pyridine. However, isocannabichromene-C_5 (IV) was prepared by refluxing olivetol with citral in pyridine at 110° for 7 hr following literature procedures (3, 4). Purification was carried out in all cases by column chromatography on silica gel with benzene–chloroform (1:1) as the solvent system.

Because cannabinoids often have been misidentified, all natural and synthetic cannabinoids were subjected to GLC and GLC–mass spectrometric analysis using electron voltage–mass fragment intensity graphs for positive identification. Trimethylsilyl derivatives were used when needed. All analyses were conducted in accordance with published protocols (5, 6).

Compounds I–V were tested using the red cell stabilization assay, and I and II also were tested using the rat paw edema assay. All testing was conducted using previously described methods (1). All of the test compounds and phenylbutazone (used as a positive control) were tested in the red cell stabilization assay at a final concentration of 1×10^{-4} *M*. Compounds I and II and phenylbutazone were tested in the rat paw edema assay at the intraperitoneal doses indicated in Table I. The intraperitoneal route was chosen because our previous work showed that cannabichromene is more effective by the intraperitoneal route than by the conventional oral route. Positive and negative controls were tested to ensure that the effects observed were true anti-inflammatory effects rather than the result of counterirritation.

Table II—Effectiveness of Cannabichromene Homologs on the Red Cell Stabilization Assay (n = 3) [a]

Compound	Inhibition, %	Mean[b]	SEM
Vehicle control	—	13.43	0.13
I	94	0.76	0.05
II	86	1.86	0.13
Phenylbutazone	66	4.59	0.11
Vehicle control	—	17.21	0.52
I	79	3.66	0.52
IV	75	4.27	0.25
III	69	5.34	0.75
V	43	9.86	0.53
Phenylbutazone	54	7.86	0.70

[a] All compounds were tested at a final concentration of 1×10^{-4} *M*. [b] Mean of three replicates of blood taken from the same dog. Compounds I and II were tested using blood from one dog, and I and III–V were tested using blood from a second dog. Vehicle positive controls from both tests are given.

Cannabichromene and its derivatives stabilized red cell membranes, as indicated by the inhibition of heat-induced hemolysis (Table II). They inhibited hemolysis more effectively than did phenylbutazone at equimolar concentrations, except for V. Both I and II inhibited carrageenan-induced rat paw edema at all doses tested. The percent inhibition produced by I and II was roughly equivalent to the inhibition produced by phenylbutazone when all were tested at 120 mg/kg. Phenylbutazone was not tested at 240 and 480 mg/kg since it is lethal at those doses.

The length and position of the side chain on the cannabichromene moiety appear to have little or no effect on its potency, with the possible exception of V. This indication is of interest since Hollister (7) found that the psychological effects of another *Cannabis* compound, (−)-*trans*-Δ^9-tetrahydrocannabinol, were diminished as the length of the side chain was reduced. For example, the C-3 side-chain homolog of (−)-*trans*-Δ^9-tetrahydrocannabinol was only ~25% as active as (−)-*trans*-Δ^9-tetrahydrocannabinol itself.

Further experiments on the anti-inflammatory effects of cannabichromene are in progress, and the results will be reported.

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