hydrates could be air milled, the author proceeded to prepare suspensions with air-milled hydrate to see if crystal growth still occurred. Work was done with I-hydrate, because at that time development work on II had been discontinued. A 2.5% suspension of air-milled I-hydrate was made (Table III) and observed for 2 years by microscopic examination and particle-size analysis. No evidence for crystal growth was observed.

This case history shows that suspensions are dynamic systems and that it is important to establish which forms are present at equilibrium. It shows that careful work is needed to identify these forms and prove that they can be processed to specifications prior to suspension formulation work. In addition, this report shows that satisfactory suspensions can be made with these equilibrium forms.

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Bicyclic Thiadiazoles I: 2-(2-Thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole

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Abstract □ 2-(2-Thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole was synthesized and evaluated for its diuretic action. The effect of 2-substitution on the ease of cyclization to a bicyclic system was studied. The carboxylic acid hydrazides were converted to dithiocarbazates which, on cyclization, gave 2-substituted 5-thio-1,3,4-thiadiazoles. Thiothiadiazoles were converted to the bicyclic system by the action of phenacyl bromide.

Keyphrases □ Thiadiazoles, bicyclic—synthesis of 2-(2-thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole, evaluated as a potential diuretic □ 2-(2-Thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole—synthesis, screened as potential diuretic □ Diuretics, potential—synthesis and screening of 2-(2-thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole

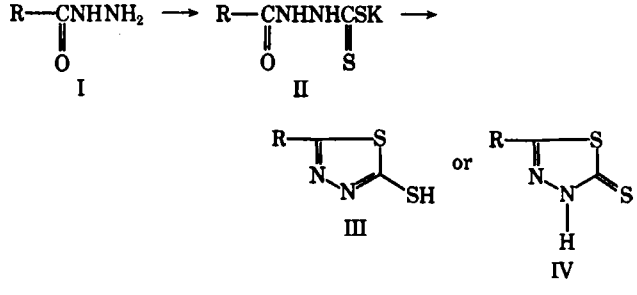
Thiazole and thiadiazole derivatives have been used successfully as diuretics (1–4). Synthesis of 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide) by Roblin and Clapp (1) led to many acetazolamide derivatives as potential diuretics (5, 6). Heterocyclic mono- and disulfonamides, particularly thiophene and benzothiophene analogs, thiazole and benzothiazole analogs, and thiazides have proved useful as diuretics (7).

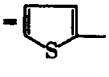
These facts prompted us to synthesize a bicyclic thiadiazole to see the effect of the condensed thiadiazole and thiazole nucleus on diuretic activity. The compound synthesized exhibited significant diuretic activity.

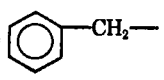
DISCUSSION

Treatment of 2-thiophenecarboxylic acid hydrazide (I*a*) and phenylacetic acid hydrazide (I*b*) with carbon disulfide and potassium hydroxide gave the desired unstable potassium dithiocarbazates II*a* and II*b* (8). Without further purification, these salts were cyclized with boron trifluoride etherate (8) to give 2-(2-thienyl)-5-thio-1,3,4-thiadiazole (III*a*) and 2-benzyl-5-thio-1,3,4-thiadiazole (III*b*). The IR of Compounds III*a* and III*b* showed the presence of the (C=S) band at 7.55 μ, the (C—NH) band at 7.05 μ, and (NH) vibration at 3.5 μ, which is only compatible with thione Structures IV*a* and/or IV*b* (Scheme I).

Reaction of 1,3,4-thiadiazole-5-thiol with 2-halogeno ketones was found to be an effective route for the synthesis of a bicyclic system (V). 2-Substitution of the 1,3,4-thiadiazole nucleus had a pronounced effect on the ease of ring closure. Compound III*a* with phenacyl bromide gave 2-(2-thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole (V) using a 6-hr. reaction period (Scheme II). Under the same conditions, 2-benzyl-5-thio-1,3,4-thiadiazole (III*b*) gave an intermediate 2-benzyl-5-(phenacylthio)-1,3,4-thiadiazole (VI) (Scheme III). Increasing the reaction time to 24, 36, and 48 hr. resulted in the same product (VI). This observation can also be substantiated by the reaction of 2-furyl-5-thiol-1,3,4-thiadiazole with phenacyl bromide, which is not reported in this paper but was observed in this laboratory. This reaction also resulted in a compound



I*a*, II*a*, III*a*, and IV*a*: R = 

I*b*, II*b*, III*b*, and IV*b*: R = 

Scheme I

Table I—Urine Volume, Sodium Output, and Potassium Output at 6 Hr. following Intramuscular Injection of Compound V

Treatment	Mean Urine Volume (ml.) for Group of Five Rats	Mean Urine Electrolyte Concentration, meq./l.		Paired *t* Probabilities		
		Sodium	Potassium	Volume	Sodium	Potassium
Control	3.9	24	3.4	<0.0025	>0.15	<0.025
10 mg./kg.	5.4	31	5.0	—	—	—

having a phenacylthio side chain. Attempts to cyclize VI with phosphoryl chloride in xylene were unsuccessful in this system (*9*, *10*). The identity of Compound V was established by NMR. The chemical shifts of C_6 and C_5 protons are δ 7.10 (s) and 2.50 (s), respectively.

PHARMACOLOGICAL EVALUATION

The diuretic activity of 2-(2-thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole (V) was studied in male Sprague-Dawley rats (165–190 g.) by measurement of the effect on the volume of urine and the urinary output of sodium and potassium. The test compound was suspended in 5% acacia and administered intramuscularly to five rats at a dose of 10 mg./kg. in a volume not exceeding 0.2 ml. Animal subjects were fasted for 18 hr. prior to use and were given a 5-ml. injection of 0.9% saline intraperitoneally immediately before the drug injection. They were housed individually in metabolism cages, and urine was collected in volumetric vessels. Levels were recorded at 60-min. intervals, and at the end of 6 hr. the final urine volumes were recorded and the sodium and potassium concentrations were determined by flame photometer. Control levels of urine volume and electrolyte output were recorded for each experimental rat prior to use in the evaluation of the experimental compound. Table I presents data from the studies described above.

Interval measurements of urine volume indicated that diuretic action began at approximately 4 hr. All mean values (volume, sodium, and potassium) were above control levels at 6 hr. Paired *t* tests, however, did not indicate statistical significance in the sodium output change.

EXPERIMENTAL[1]

2-(2-Thienyl)-5-thio-1,3,4-thiadiazole (IIIa)—To a solution of 2-thiophenecarboxylic acid hydrazide (*Ia*) (11.4 g., 0.08 mole) and potassium hydroxide (9.8 g.) in methanol (160 ml.) was added carbon disulfide (10 ml.). The solution was stirred at room temperature for 5 hr., diluted with cold ether (800 ml.), and cooled in the refrigerator for 2 hr. It was then filtered. The precipitate was washed with cold ether and dried. Without further purification, the potassium dithiocarbazate (*IIa*) [yield 15.2 g. (75%), m.p. 284–285°] was suspended in methylene chloride (750 ml.) under a nitrogen atmosphere. After 80 ml. of boron fluoride ethyl ether was added, the mixture was stirred for 18 hr., poured onto a mixture of ice and water, and extracted with ether. The ether layers were combined and extracted with potassium hydroxide (10%). The alkaline solution was acidified with cold hydrochloric acid (10%). A white precipitate formed which was filtered, washed with water, and dried. The crude product, on recrystallization from benzene, yielded 2.1 g. (17%) of analytical sample, m.p. 193–194°. The IR spectrum (KBr) showed absorptions at 6.50 and 6.70 (typical of thiadiazole), 7.55 (C=S), 7.05 (C—NH), and 3.50 (NH) μ.

Anal.—Calc. for $C_6H_4N_2S_2$: C, 35.97; H, 2.01; N, 13.98. Found: C, 36.02; H, 2.12; N, 13.83.

Similarly, 2-benzyl-5-thio-1,3,4-thiadiazole (*IIIb*) was obtained in 30% yield. Recrystallization from benzene afforded needles, m.p. 118–119°; IR (KBr): 6.40, 6.60, 7.60, and 7.00 μ.

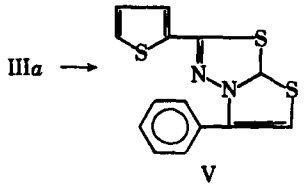
Anal.—Calc. for $C_9H_8N_2S_2$: C, 51.89; H, 3.87; N, 13.45. Found: C, 51.80; H, 3.87; N, 13.62.

2-(2-Thienyl)-5-phenylthiazolo[2,3-*b*]-1,3,4-thiadiazole (V)—A solution of *IIIa* (1.0 g., 0.005 mole) and phenacyl bromide (1.0 g., 0.005 mole) in absolute ethanol (40 ml.) was refluxed for 6 hr. and left at room temperature for 3 days. Light-pink needles separated. Recrystallization from benzene afforded an analytical sample, m.p. 134–135°, yield 0.5 g. (32%); IR (KBr): 6.00, 6.30, 6.80, and 6.95 μ.

Anal.—Calc. for $C_{14}H_{10}N_2S_2$: C, 55.59; H, 3.33; N, 9.26. Found: C, 55.30; H, 3.23; N, 9.01.

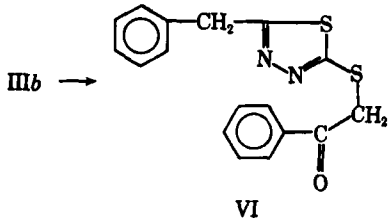
2-Benzyl-5-(phenacylthio)-1,3,4-thiadiazole (VI)—A procedure similar to the one used for Compound V was followed. The reaction time was increased to 36 hr. The crude material, on recrystallization from benzene, gave a pure sample, m.p. 105–106°, yield 80%; IR (KBr): 5.80 (C=O), 6.30 (C=N), 6.40, and 6.75 μ.

Anal.—Calc. for $C_{17}H_{14}N_2OS_2$: C, 62.54; H, 4.32; N, 8.58. Found: C, 62.48; H, 4.10; N, 8.56.



V

Scheme II



VI

Scheme III

[1] Reported melting points are uncorrected. A Thomas-Hoover Uni-Melt apparatus was used for melting-point determinations. Galbraith Laboratories, Inc., Knoxville, Tenn., conducted the elemental analyses. IR spectra were taken on Perkin-Elmer model 137-B Infracord spectrophotometer. The NMR spectra were taken on Varian XL-100 NMR spectrometer.

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