Labile Triazinoindole Hydrates

H. C. CALDWELL

Abstract □ Aqueous topical suspensions of two triazinoindoles in fine particle form produced crystals even when the protective colloid methylcellulose was added. These crystals were found to be monohydrates that readily lost water of hydration under mild heat, but they could be air milled with no water loss. One hydrate was formulated in suspension and found to be free of crystal growth for 2 years.

Keyphrases □ Triazinoindole hydrates, labile—formation, isolation from topical suspensions, effects of air milling and methylcellulose on crystal growth □ Hydrates, labile—formation, isolation from topical triazinoindole suspensions, effects of air milling and methylcellulose on crystal growth □ Suspensions of labile triazinoindole hydrates—formation, isolation, effects of air milling and methylcellulose on crystal growth

Over 90 hydrates are included in USP XVIII and NF XIII. Four of these that readily lose water of hydration in warm dry air are atropine sulfate monohydrate, morphine sulfate pentahydrate, scopolamine hydrobromide trihydrate, and sodium acetate trihydrate. These, like other official hydrates, are usually stored in tight, light-resistant containers.

According to the USP and NF, a tight container protects the contents from contamination by extraneous liquids, solids, or vapors; from loss of the drug; and from efflorescence, deliquescence, or evaporation under ordinary or customary conditions of handling, shipment, storage, and distribution. It is also capable of tight reclosure. Thus, official hydrates properly packaged and stored should not lose moisture. Further, it is reasonable to assume that these hydrates exist as such in formulations.

It is important to recognize that many official compounds form hydrates that readily lose water when dried under mild conditions. The review (1) on hydrates of alcohols and glycols is illustrative. These labile hydrates become particularly important when aqueous suspensions of slightly soluble, anhydrous chemicals are formulated, because these hydrates can produce large particles as they form (2). These large particles would have less surface area for a given dose, and the rate and extent of absorption could be markedly decreased (3). Protective colloids might be expected to retard but not stop this transformation (2, 4).

This report presents a case history of suspension work on two triazinoindoles (I[1] and II[2]) and their labile hydrates. Ravin *et al.* (4) recently described the physical-chemical evaluation of I. They reported that it is practically insoluble (0.008 mg./ml.), that it exists as two polymorphs, and that it forms a hydrate.

The present tasks were to prepare and to evaluate formulations for oral and nasal use. The most stable polymorph of I was used, but it was not known then that I or II formed hydrates.

EXPERIMENTAL

Particle-Size Reduction—The purified compounds were passed through a fluid energy mill[3]. The products were sized with the Coulter counter[4].

Suspensions—The milled chemicals were suspended in buffered vehicles containing a preservative and a protective colloid.

Hydrate Preparation—Small batches of I-hydrate and II-hydrate were made by suspending 5 g. of air-milled I and II in 750 ml. of distilled water and heating the suspensions overnight on the steam bath. The small anhydrous crystals were converted to long hydrate needles. Larger batches of I-hydrate were made by dissolving I in dilute hydrochloric acid and precipitating it with dilute sodium hydroxide solution. The suspension was stirred overnight at room temperature. Samples were collected and air dried at room temperature.

X-Ray Diffraction Procedure—The procedure employed was described (4) previously but was modified so that intensities were not measured by recording the time necessary to count a fixed number of particles.

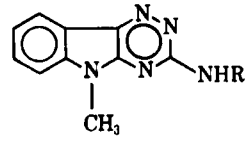
Thermal Work—A differential thermal analyzer[5] and a thermogravimetric analyzer[6] were used. Heat rates were 20 and 5°/min., respectively. A thermobalance[7] was used to follow weight loss as a function of temperature.

Isolation of Hydrates from Topical Suspensions—Suspensions made with I and II were followed for chemical and physical changes at various storage conditions. Samples that appeared, by microscopic examination, to have completely changed from small to large particles were diluted with water, filtered, washed, and dried at room temperature.

RESULTS AND DISCUSSION

Suspensions of air-milled anhydrous I and II were found to produce large crystals even in the presence of classical crystal growth inhibitors such as methylcellulose. To determine if hydrate formation was responsible, it was necessary to isolate large crystals from I and II suspensions and compare them to crystals of authentic hydrates of I and II.

These data are reported in Tables I and II. Both hydrates of I and both hydrates of II (Table II) analyze as monohydrates. In the thermal gravimetric analysis, all hydrates start to lose weight at relatively low temperature and each loses weight equivalent to 1 mole of water. As expected, the anhydrous forms do not lose weight. In the differential thermal analysis, the hydrate of I shows transitions at about 108–117°, which are not seen with the anhy-



I: R = $(CH_2)_2$—$C(CH_3)_2$—OH
II: R = $(CH_2)_3$—OH

[1] SK&F 30097.
[2] SK&F 21687.

[3] Trost jet mill, Helme Products, Inc., Helmetta, N. J.
[4] Model B.
[5] Du Pont 900.
[6] Du Pont 950.
[7] Perkin-Elmer TGS-1.

Table I—Thermal Gravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) Studies on I and II and Their Hydrates

Number	Compound	Description	—Weight Loss by TGA— Onset of Loss	End of Loss	Loss, %	DTA, Transitions				
1	I	Air-milled base	—	—	0	—	—	171°	—	192.5°
2	I-hydrate	Isolated from topical suspension, air dried at room temperature	34°	115°	5.9[a]	117°	—	168°	—	190°
3	I-hydrate	Air-milled No. 2	52°	96°	5.7	108°	—	167.5°	—	189°
4	I-hydrate	Prepared in warm water, air dried at room temperature	50°	78°	5.9	112.5°	—	169°	—	191°
5	I-hydrate	Air-milled No. 4	57°	88°	5.9	111.5°	—	170°	—	192°
6	II	Air-milled base	—	—	0	23[b]	—	—	—	164.5°
7	II-hydrate	Isolated from topical suspension, air dried at room temperature	32°	118°	6.35[c]	23[b]	—	105°	125°	163°
8	II-hydrate	Prepared in warm water, air dried at room temperature	32°	104°	6.75	23[b]	100°	107.5°	126°	163°

[a] 5.95% theoretical for monohydrate. [b] Exotherm—others are endotherms. [c] 6.54% theoretical for monohydrate.

drous form, and the hydrate of II shows transitions at about 105 and 125°, which are not seen with the anhydrous form. Thus, these data show that suspensions of I and II form hydrates even in the presence of protective colloids. X-ray diffraction patterns for II and II-hydrate are distinctly different, as Fig. 1 shows. X-ray diffraction patterns for I and I-hydrate were reported (4) to be different previously. Weight loss as a function of temperature was determined. Samples were placed in the thermobalance, and percent weight loss was recorded after 15 min. at various temperatures. I-hydrate (air milled) lost no weight at 45°, 1% at 50°, 4% at 55°, and all (5.95%) at 60°. II-hydrate lost no weight at 25°, 0.8% at 28°, 1.5% at 33°, 6.2% at 45°, and all (6.54%) at 60°. Hydrous lactose USP lost no weight at 45° and 1% at 60°, while hydrous sodium citrate lost no weight at 60°.

These data show that the hydrates of I and II are heat labile, and they explain why hydrate samples dried at 60° would be rapidly converted to anhydrous forms.

Because the anhydrous forms of I and II were converted to hydrates in suspension, it was reasonable to think that suspensions of hydrates would be stable with respect to crystal growth. But one other problem had to be solved before suspension work could begin. It had to be established that the hydrates of I and II could be air milled with no water of hydration loss. Three batches of I-hydrate were passed through the air mill with no water loss, as determined by Karl Fischer titration and weight loss on drying. Two batches of II-hydrate treated in the same manner also did not lose water.

Once it had been established that the large crystals formed in suspensions of I and II were the respective hydrates and that the

Table II—Elemental Analysis of Hydrates

Number[a]	Compound	Description		—Analysis, %— Calc.	Found
2	I-hydrate	Isolated from topical suspension, air dried at room temperature	C	59.39	59.45
			H	6.98	6.92
			N	23.09[b]	23.02
4	I-hydrate	Prepared in warm water, air dried at room temperature	C	59.39	59.25
			H	6.98	6.89
			N	23.09[b]	23.09
7	II-hydrate	Isolated from topical suspension, air dried at room temperature	C	56.72	56.82
			H	6.22	6.26
			N	25.44[c]	25.44
8	II-hydrate	Prepared in warm water, air dried at room temperature	C	56.72	56.92
			H	6.22	6.28
			N	25.44[c]	25.61

[a] Numbers are from Table I. [b] Calculated for $C_{15}H_{19}N_5O_2 \cdot H_2O$. [c] Calculated for $C_{13}H_{15}N_5O \cdot H_2O$.

Table III—I-Hydrate Suspension

Ingredients	% w/w
I-hydrate (air milled)	2.658[a]
Methylcellulose[b]	1.000
Sodium citrate USP	0.200
Potassium biphthalate, reagent	0.130
Eucalyptol	0.020 v/v
Thimerosal NF	0.001
Sodium chloride USP	0.810
Water for injection, *q.s.* ad	100.000
pH	5.4
Freezing-point depression	0.61°

[a] Equivalent to 2.500% anhydrous I. [b] Methocel, Type MC Premium 15 cps.

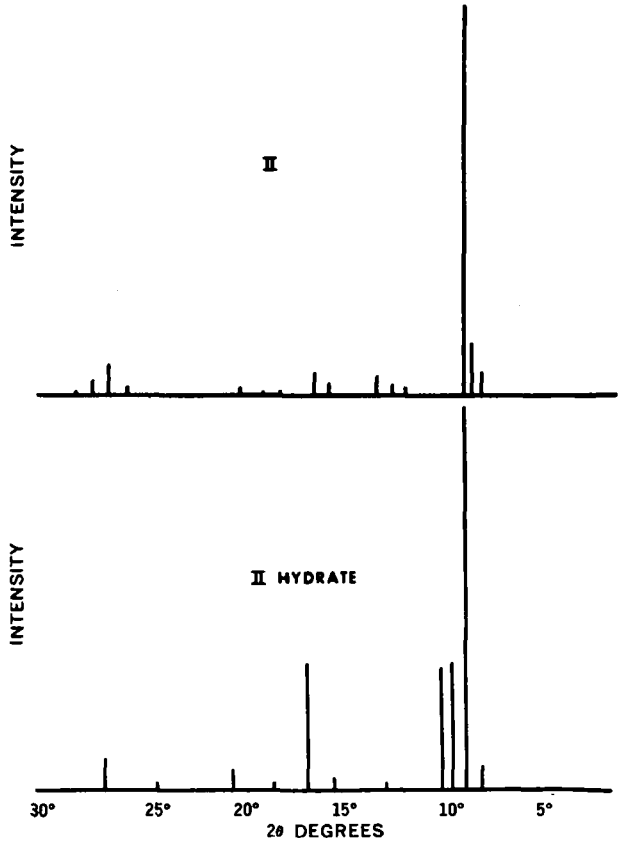


Figure 1—*X-ray diffraction patterns for anhydrous and hydrate forms of II.*

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

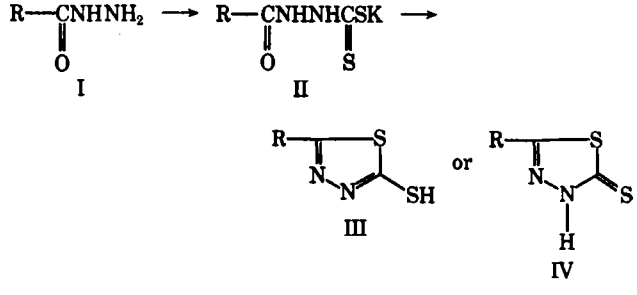
Thiazole and thiadiazole derivatives have been used successfully as diuretics (1–4). Synthesis of 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide) by Roblin and Clapp (1) led to many acetazolamide derivatives as potential diuretics (5, 6). Heterocyclic mono- and disulfonamides, particularly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

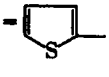
These facts prompted us to synthesize a bicyclic thiadiazole to see the effect of the condensed thiadiazole and thiazole nucleus on diuretic activity. The compound synthesized exhibited significant diuretic activity.

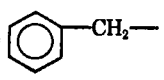
DISCUSSION

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