Synthesis of Methyl 1-(3,4-Dichlorobenzyl)-hexahydro-2,3-dioxo-4-pyridinecarboxylate

THOMAS J. SCHWAN ˣ, JOSEPH E. GRAY, and GEORGE C. WRIGHT

Received May 23, 1978, from the *Research Department, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication September 18, 1978.

Abstract □ Methyl 1-(3,4-dichlorobenzyl)-hexahydro-2,3-dioxo-4-pyridinecarboxylate was prepared by alkylation of methyl hexahydro-2,3-dioxo-4-pyridinecarboxylate with α,3,4-trichlorotoluene. The compound showed significant antifungal activity when challenged by a spectrum of pathogenic yeast species in a minimum inhibitory concentration test. It was active against some dermatophytic fungi and *Aspergillus niger.*

Keyphrases □ Methyl 1-(3,4-dichlorobenzyl)-hexahydro-2,3-dioxo-4-pyridinecarboxylate—synthesized and screened for antifungal activity □ Antifungal activity—synthesis and screening of methyl 1-(3,4-dichlorobenzyl)-hexahydro-2,3-dioxo-4-pyridinecarboxylate

The literature contains no reports of *N*-substituted benzyl hexahydro-2,3-dioxo-4-pyridinecarboxylates. Such a compound was desired for antifungal evaluation. Accordingly, the methyl ester (I) was treated with α,3,4-trichlorotoluene (II) in toluene in the presence of 1 *M* equivalent of sodium hydride. No crystalline product resulted, and it is possible that a mixture of products was obtained by C- or *O*-alkylation of the monoanion (III) (Scheme I).

For this reason, I was treated with 2 *M* equivalents of sodium hydride, presumably to generate the dianion (IV). Alkylation of IV with 1 *M* equivalent of II generated the 1-benzyl enolate ion (V). Acidification of V gave rise to VI (Scheme II).

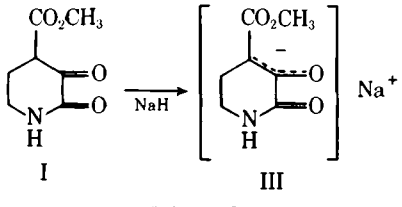
Alkylation of I in this manner gave VI in yields ranging from 9 to 21% (Scheme II). The structural assignment of VI is based on IR spectral evidence, elemental analysis, and the NMR spectrum, which is highlighted by a singlet at δ 4.61 ppm, indicative of the *N*-benzyl moiety.

DISCUSSION

The antifungal activity of VI was challenged by a spectrum of yeast species in a minimum inhibitory concentration procedure (1) in Sabouraud's liquid medium BBL. *Candida albicans* (Eaton Code Nos. VM-71, VM-81, and M-3), *Candida krusei* (VM-29B), *Candida guilliermondi* (VM-42), and *Torulopsis glabrata* (VM-22) were inhibited at 20 μg/ml; *Candida tropicalis* (VM-25) was inhibited at 30 μg/ml. The fungistatic activity of VI was determined by an agar dilution procedure (2) using Sabouraud's dextrose agar BBL.

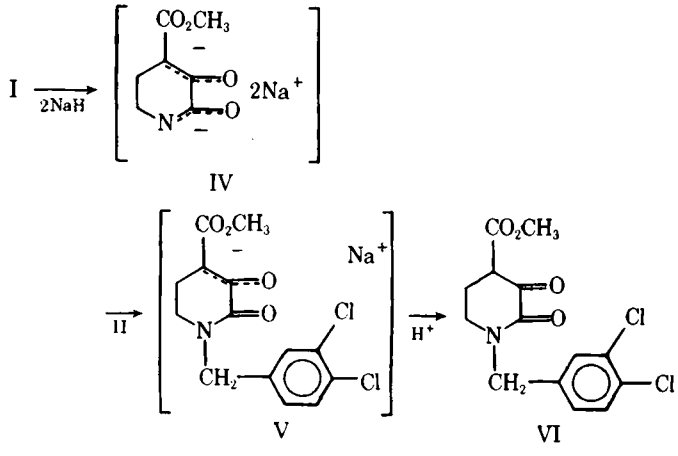
Plates inoculated with *Microsporum canis* (M-4) and *Aspergillus niger* (M-29) were incubated at 25°. Complete inhibition of growth for 10 days was the criterion for activity. Under these conditions, *M. canis* was inhibited by 10 μg/ml and *A. niger* was inhibited by 100 μg/ml.

Fungicidal activity of VI was determined by exposing viable cultures



I III

Scheme I



Scheme II

of dermatophytic organisms in agar plugs to a 2% solution of VI in polyethylene glycol 400 for specific time intervals (3). After exposure, the plugs were rinsed and subcultured. Cultures of *M. canis* (M-4), *Microsporum audouini* (M-17), *Trichophyton mentagrophytes* (M-93), and *Trichophyton tonsurans* (M-76) were sterilized by exposure of 15 min or less. Compound VI was significantly active against yeast and mold species.

EXPERIMENTAL[1]

Methyl hexahydro-2,3-dioxo-4-pyridinecarboxylate (I) was prepared by transesterification of ethyl hexahydro-2,3-dioxo-4-pyridinecarboxylate (4), using sodium methoxide in methanol. Recrystallization from methanol gave an analytical sample, mp 155–156°; IR: 3.05 (NH), 5.91 (C=O ester), 6.02 (C=O amide), and 8.05–8.15 (COC ester) μm.

Anal.—Calc. for $C_{11}H_{18}N_2O_5$: C, 49.12; H, 5.30; N, 8.18. Found: C, 49.02; H, 5.53; N, 8.07.

Methyl 1-(3,4-dichlorobenzyl)-hexahydro-2,3-dioxo-4-pyridinecarboxylate (VI) was prepared as follows. To a mixture of 51.3 g (0.30 mole) of I in 1000 ml of toluene was added 24 g of sodium hydride (60%) in mineral oil (*i.e.*, 14.4 g, 0.60 mole). The mixture was stirred and refluxed for 1.5 hr and cooled to room temperature, and 58.5 g (0.30 mole) of II was added rapidly. Then the mixture was stirred and refluxed for 18 hr and cooled to 15–20°, and 2.0 ml of methanol and 30 ml of acetic acid were added. With the temperature maintained at 10–15°, the mixture was diluted with 800 ml of water.

The toluene layer was separated, and the aqueous layer was extracted with 300 ml of toluene. The combined organic layers were washed with 500 ml of water, dried (magnesium sulfate), and concentrated to dryness *in vacuo*. The oily residue was washed with 3 × 100 ml of cold hexane and crystallized from 125 ml of toluene to give 9.40 g (9.5%) of the product, mp 94–99°.

Further recrystallization from toluene gave the analytical sample, mp 99–101°; IR: 5.92 (ester C=O), 6.00 (amide C=O), 8.00 (COC ester), and no NH at 3.00–3.30 μm; NMR (deuterochloroform): δ 2.56 (t, 2, pyridine, 5-CH_2), 3.36 (t, 2, pyridine, 6-CH_2), 3.86 (s, 3, OCH_3), 4.61 (s, 2, NCH_2Ar), 7.01–7.20 (m, 3, phenyl CH), and 11.10 (m exchanges with D_2O, 1, pyridine, 4-CH) ppm.

[1] Melting points were determined on a Mel-Temp apparatus, and those below 230° are corrected. IR spectra were determined as mineral oil mulls with a Perkin-Elmer 137B spectrophotometer. NMR spectra were obtained on a Varian A-60A instrument with tetramethylsilane as an internal standard.

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Anal.—Calc. for $C_{14}H_{13}Cl_2NO_4$: C, 50.93; H, 3.98; N, 4.24. Found: C, 51.10; H, 4.02; N, 4.27.

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Rapid, Stability-Indicating, High-Pressure Liquid Chromatographic Determination of Theophylline, Guaifenesin, and Benzoic Acid in Liquid and Solid Pharmaceutical Dosage Forms

D. R. HEIDEMANN

Received August 21, 1978, from the *Analytical Research Department, Dorsey Laboratories (Division of Sandoz, Inc.), Lincoln, NE 68501.* Accepted for publication September 29, 1978.

Abstract □ Theophylline, guaifenesin, and benzoic acid were determined by reversed-phase high-pressure liquid chromatography without interference from active and/or vehicle decomposition. A degradation product of sucrose, 5-hydroxymethylfurfural, can be identified and quantified in liquid samples simultaneously.

Keyphrases □ Theophylline—analysis, high-pressure liquid chromatography, in liquid and solid pharmaceutical dosage forms, stability □ Guaifenesin—analysis, high-pressure liquid chromatography, in liquid and solid pharmaceutical dosage forms, stability □ Benzoic acid—analysis, high-pressure liquid chromatography, in liquid and solid pharmaceutical dosage forms, stability □ High-pressure liquid chromatography—analysis of theophylline, guaifenesin, benzoic acid in liquid and solid pharmaceutical dosage forms

Determination of active components and preservatives in pharmaceutical products subjected to aging and stress requires a highly specific method. The actives and preservatives must be determined in the presence of vehicle degradation as well as their degradation products.

Vehicle degradation is particularly critical in syrups, where the decomposition of hexose sugars results in a series of complex reaction products (1–3). Resulting UV-absorbing species, such as 5-hydroxymethylfurfural, can interfere with some assays (4).

Theophylline and guaifenesin, widely used in asthmatic preparations, have been assayed by various methods. Spectrophotometric determinations are rapid but nonspecific unless the actives are separated from interfering species adequately (5–7).

While GLC methods demonstrate greater specificity, derivatization is generally required to avoid excessive tailing due to the polar nature of theophylline and guaifenesin (8–15). In addition, theophylline sodium glycinate, a commonly used water-soluble form of theophylline, is not readily soluble in organic solvents required by GLC methods.

Determination of theophylline and guaifenesin by high-pressure liquid chromatography (HPLC) in biological

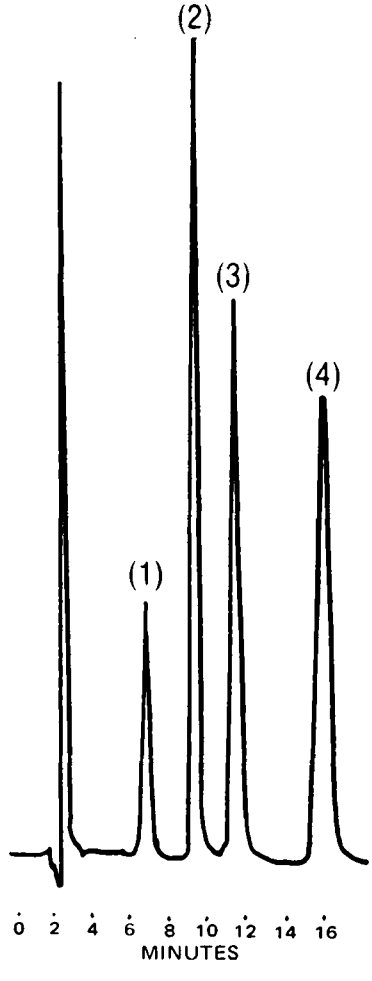

Figure 1—*Representative chromatogram of an elixir. The concentrations of components in the sample were: (1), benzoic acid, 0.01 mg/ml; (2), theophylline sodium glycinate, 0.2 mg/ml; (3), guaifenesin, 0.067 mg/ml; and (4), methylparaben internal standard, 0.1 mg/ml.*

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