Table I—pH Levels of Isopropyl Myristate Prepared by Three Methods and Corresponding D Values of *Pseudomonas aeruginosa*

Method	pH	D Value, min: Room Temperature	D Value, min: 47°
USP XX Column-filtered			
A	6.7	>60.0	8.0
B	6.6	>60.0	9.3
Batch-Swirl			
A	6.7	>60.0	12.1
B	6.3	>60.0	11.6
Untreated			
A	4.0	11.4	<10.0
B	3.7	10.0	<10.0

Table II—Comparison of Flow Rates for Membrane Filters Used in the Filtration of Oils and Ointments

Membranes	Flow Rate, min[a]: Isopropyl Myristate 0.2 μm[b]	Isopropyl Myristate 0.45 μm[b]	Fluids A and D 0.2 μm[b]	Fluids A and D 0.45 μm[b]
Acetate Cellulose	2	1	3	1
Cellulose Nitrate	3	1	>120	20
Polyester	2	1	5	2
Mixed Esters of Cellulose	3	1	83	2
Polycarbonate	4	1	14	3
Mixed Cellulose Acetate–Pyroxylin	3	1	>120	19

[a] Approximate time. [b] Pore size of membrane.

RESULTS

The pH levels of the untreated isopropyl myristate, the USP XX column isopropyl myristate, and the batch-swirled isopropyl myristate are shown in Table I. The D values of *P. aeruginosa* obtained at room temperature and at 47°, which correspond to the three methods of preparation of isopropyl myristate, are also shown.

The approximate time required to filter isopropyl myristate and the rinsing fluids, A and D, through various membranes are given in Table II. The acetate cellulose membrane[4] and the polyester membrane[5] showed the fastest flow rates for the 0.2-μm membrane: 2 min for isopropyl myristate with both filters and 3 and 5 min, respectively, for rinsing fluids A and D. An oily film was noted on the polyester membrane after filtration of the isopropyl myristate and rinsing fluids A and D, regardless of pore size.

The flow rates of both the 0.2-μm mixed cellulose acetate–pyroxylin and the pyroxylin membranes exceeded 2 hr for the rinsing fluids. All of the 0.45-μm membranes had acceptable flow rates for isopropyl myristate. Flow rates for the rinsing fluids (pyroxylin, 20 min and cellulose acetate-pyroxylin, 19 min) were not acceptable.

DISCUSSION

The batch-swirl method for detoxification of isopropyl myristate used in the analysis of oils and ointments was simpler and faster than the USP XX method, *i.e.*, 1 hr *versus* 8 hr for the detoxification process. In the batch-swirl method, filter sterilization immediately followed the stirring, with no need for centrifugation. The pH of the isopropyl myristate prepared by the batch-swirl method met the requirements of the USP XX, and D values of the test organisms were comparable to those obtained by the USP XX method.

Use of cellulose acetate filters rather than 0.22- and 0.45-μm mixed cellulose acetate-pyroxylin membranes reduced the analysis time. Rapid filtration reduced the exposure time of microbial contaminants to the toxicity of isopropyl myristate and, thus, provided greater probability of their detection.

[4] Sartorius Filters, Inc., Hayward, Calif.
[5] Nucleopore, Pleasanton, Calif.

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Inc., Rockville, Md., 1980.

(2) F. W. Bowman, E. W. Knoll, M. White, and P. Mislivec, *J. Pharm. Sci.*, **61**, 532 (1972).

(3) K. Tsuji and J. H. Robertson, *Appl. Microbiol.*, **25**, 139 (1973).

(4) J. H. Robertson, *Bull. Parenter. Drug Assoc.*, 288 (1974).

(5) W. T. Sokolski and C. G. Chidester, *J. Pharm. Sci.*, **53**, 103 (1964).

(6) A. Hart and M. B. Ratansi, *J. Pharm. Pharmacol.*, **27**, 142 (1975).

(7) F. W. Bowman, in "Industrial Sterilization," G. B. Phillips and W. S. Miller, Eds., Duke University Press, Durham, N.C., 1972, p. 42.

Synthesis and Antitumor Testing of 3-Methenylthiochroman-4-one-1,1-dioxide

MARK H. HOLSHOUSER *x and LARRY J. LOEFFLER

Received July 6, 1981 from the *Division of Medicinal Chemistry, School of Pharmacy, The University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication September 18, 1981.

* Present address: Department of Medicinal Chemistry, School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.

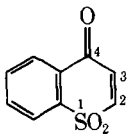
Abstract □ Treatment of thiochroman-4-one-1,1-dioxide (II) with paraformaldehyde and dimethylamine hydrochloride in isopropyl alcohol at reflux afforded directly in 89% yield the dimeric dihydropyran (IV), corresponding to the dimerization of the target compound 3-methenylthiochroman-4-one-1,1-dioxide (III). Neither the monomer III nor the expected Mannich base, 3-dimethylaminomethylthiochroman-4-one-1,1-dioxide, were isolated under conditions of the reaction. The monomer III could be prepared in 55% yield by sublimation of the dimer IV at 230–250°; however, redimerization slowly occurred at room temperature. The dimer IV was also prepared by the use of paraformaldehyde and *N*-methylanilinium trifluoroacetate. The monomer III was found to be marginally active at 10 mg/kg/day *versus* Ehrlich ascites tumor growth in mice.

Keyphrases □ Antitumor agents—3-methenylthiochroman-4-one-1,1-dioxide, synthesis, testing, dimerization, mice □ α-Methenyl carbonyl function—alkylating moiety in some antitumor agents, synthesis of 3-methenylthiochroman-4-one-1,1-dioxide

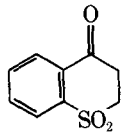
The presence of a ring system containing an α-methenyl carbonyl function is important as an alkylating moiety in some antitumor agents (1–5). So called quinone methides (1–3), α-methenyl derivatives of quinones, are thought to be active metabolic intermediates in alkylation mechanisms. In general, the stability of the α-methenyl carbonyl

0022-3549/82/0600-0715$01.00/0

function is variable, depending on substitution (3). In working with novel compounds containing the thiochromone-1,1-dioxide (I) and thiochroman-4-one-1,1-dioxide (II) ring systems (6), it became desirable to synthesize the 3-methenyl derivative (III) (Scheme I), the sulfone analog of a naphthoquinone methide, as a potential antitumor agent of the alkylating type and a possible active metabolite of previously reported compounds (6). The synthesis, testing, and dimerization of III is the subject of this study.



I



II

EXPERIMENTAL[1]

Sulfone Dimer (IV) (Method A)—Compound IV was formed by a modification of the method of Welch, *et al.* (7). A suspension of 7.76 g (39.6 mmoles) of II, 2.86 g (35.6 mmoles) of dimethylamine hydrochloride, 2.5 g (30 mmoles) of paraformaldehyde, and 0.72 ml of saturated hydrogen chloride–isopropyl alcohol solution in 25 ml of isopropyl alcohol was refluxed for 24 hr at 75°. At the end of this period a fine, white precipitate had formed. The suspension was cooled, filtered, and the solid washed with isopropyl alcohol and ether, then recrystallized from dimethylformamide. A white powder resulted with a melting point of 249–250° (yield 7.5 g, 89%).

Anal.—Calc. for $C_{20}H_{18}O_6S_2$: C, 57.39; H, 4.34. Found: C, 57.45; H, 4.22. IR: 1700 (C=O), 1300 (S=O, antisymmetrical), 1145 (S=O, symmetrical) cm^{-1}; NMR (acetone-d_6): δ 2.52 (s, 2, —C—CH_2—C—), 2.83 (s, 2, —OCH_2—), 4.10 (s, 2, 2′H), 4.39 [s, 2, 2 (II)], 7.57–8.30 (m, 8, 5–8H, 5′–8′H) ppm.

Sulfone Dimer IV (Method B)—Compound IV was also synthesized by a modification of the method of Gras (8). Paraformaldehyde (1.08 g, 12 mmoles), *N*-methylanilinium trifluoracetate (2.25 g, 10 mmoles), and (II) (1.96 g, 10 mmoles) were added to 12 ml of dioxane (dried over potassium hydroxide, then distilled from sodium metal). The mixture was refluxed for 2 hr under nitrogen, cooled, and 1.125 g (5 mmoles) of *N*-methylanilinium trifluoracetate, 0.54 g (6 mmoles) of paraformaldehyde, and 16 ml of dioxane were then added. The mixture was refluxed an additional 2 hr, allowed to cool, then extracted with chloroform, washed with a 2.5% sodium bicarbonate solution, dried over magnesium sulfate, and evaporated. The resulting white powder was dissolved in dimethyl sulfoxide and crystals formed as water was added. The product was collected and dried *in vacuo* at 100°, yielding 500 mg, 30%.

3-Methenylthiochroman-4-one-1,1-dioxide (III)—Compound IV, the dimer, (200 mg, 0.48 mmole) was placed in a sublimation apparatus and sublimed at 230–250° (1 mm Hg). On the condenser appeared a mixture of Compounds III and IV. Compound III could be purified by either repeated sublimation or column chromatography on silica gel eluted with chloroform, the monomer III eluting before the dimer IV. On immediate solvent evaporation and freezing in a desiccator, compound III remains relatively stable; yield: 110 mg (55%) observed mp 248–250°. TLC: silica gel, $CHCl_3$, R_f = 0.26. IR: 1699 (C=O), 1665 (C=C, conjugated), 1300 (S=O, antisymmetrical), 1145 (S=O, symmetrical) cm^{-1}; NMR ($CDCl_3$): δ 4.42 (s, 2,2H), 5.89 (d, 1, J = 1 Hz, =CH_2), 6.65 (d, 1, J = 1 Hz, =CH_2), 7.5–8.32 (m, 4, 5–8H) ppm.

***In Vivo* Ehrlich Ascites Screen**—All test compounds were homogenized in 0.05% polysorbate 80 and administered in doses ranging from 5 to 20 mg/kg/day ip. Tumor cells (2×10^6) were injected into CF_1 male mice (~25 g) on day 0 (n = 6). Test compounds were administered intraperitoneally on days 1–8. On day 9 the mice were sacrificed and peritoneal ascites cell volume and packed cell volume (ascites-crit) were determined by the method of Piantadosi *et al.* (9). Results of this screen are reported as percent inhibition calculated by the following:

$$\% \text{ inhibition} = 100 - \left[\frac{(\text{vol of treated})(\text{ascrit of treated})}{(\text{vol of control})(\text{ascrit of control})}\right] \times 100 \quad \text{(Eq. 1)}$$

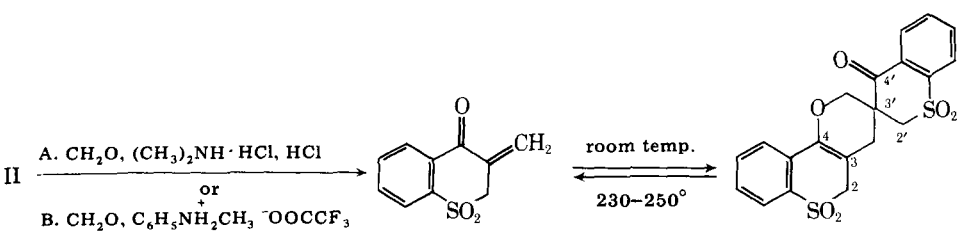
6-Mercaptopurine was used as an internal standard. Six to eight mice were used per test compound, and >80% inhibition was considered significant.

RESULTS AND DISCUSSION

The classical synthetic approach to compound III involves Mannich base formation alpha to the carbonyl of II followed by base-catalyzed amine elimination. However, attempts to form the Mannich base (7) of II using dimethylamine hydrochloride and paraformaldehyde under acidic conditions (*i.e.*, the acid-catalyzed enol of II serving as a nucleophile) afforded an insoluble high-melting neutral product which was identified by NMR as the dihydropyran type dimer, IV (Scheme I). Compound IV resulted from the initially formed III, the desired product, which was formed either by spontaneous amine elimination (7) from the Mannich base or direct reaction with the hydroxycarbenium ion of protonated formaldehyde with no involvement of the amino species (10). Subsequently, a Diels-Alder type 1,4-addition of one molecule of III to the exomethylene group of another molecule of III occurred, forming the dihydropyran ring system of IV. High temperature was required to reverse the dimerization. Formation and subsequent isolation of III was accomplished by sublimation of IV at 230–250° (1 mm Hg), followed by rapid column chromatography of the material appearing on the condenser. Good yields of IV were obtained from II (89%), however, a poorer yield of III (55%) resulted in the reversal step. Furthermore, at room temperature and/or in the presence of solvent, III redimerized completely within 48 hr. The identical observed melting points for III and IV (248–250°) resulted from redimerization of III on slow heating, as indicated by TLC.

Specific reagents for preparing α-methenyl ketones provided an alternate method for the synthesis of III (8, 11, 12). Use of one of these agents, *N*-methylanilinium trifluoracetate (8) afforded IV as well. In anticipation of the same result, no other reagent was tried. It was of interest that this 3-exomethylene derivative of III is isolable in the thiochroman-4-one-1,1-dioxide series. This appeared not to be the case with the naphthoquinone methides previously generated, which required trapping (3). Isolability of such a reactive intermediate as III opens a route to the synthesis of a wide variety of novel 3-substituted thiochroman-4-one-1,1-dioxides *via* Michael, Diels-Alder, or 1,3-dipolar type reactions.

Freshly prepared and analyzed compound III, immediately tested at



Scheme I

[1] All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. TLC was performed on precoated plates (silica gel 60-F-254, EM reagents) with fluorescent backing. The plates were visualized by UV light. Elemental analyses were done by Atlantic Microlab, Atlanta, Ga., and agreed with theoretical values within ±0.4%.

Infrared spectra were obtained in NUJOL using a Perkin-Elmer Model 297 IR spectrophotometer. Photon NMR spectra were taken on a JEOLCO JNM-FX60 using tetramethylsilane as an internal standard. All IR and NMR spectra were completely consistent with assigned structures. Starting materials were used as received from suppliers unless otherwise indicated.

Table I—Effects of Test Compounds on Ehrlich Ascites Carcinoma[a] Growth

Compound	Dose, mg/kg/day ip	Survival at Day 9	Ascrit[b]	Ascites Volume, Mouse	Inhibition[c], %
0.05% Polysorbate 80		34/40	33.6 ± 8.7	1.8 ± 1.02	0.0
V[d]	5	8/8	22.0	2.45	57.1
	10	8/8	0.0	0.0	100.0
	20	8/8	35.7	0.88	81.8
III	10	8/8	43.8	0.15	80.0
IV	10	6/6	21.2	2.2	0.0
6-Mercaptopurine[e]	200	6/6	0.3	0.7	99.6

[a] 2×10^6 cells were injected intraperitoneally into 6 or 8 male CF_1 mice on day 0. The drug was administered from day 1 to 8. On day 9 the mice were sacrificed and the experiment was evaluated. [b] Packed cell volume as a percent. [c] Greater than 80% inhibition is required for significant activity. [d] Compound V is 3-chloromethylthiochromone-1,1-dioxide (6). [e] Sigma Chemical Co.

10 mg/kg/day in mice, *versus* Ehrlich ascites carcinoma tumor growth, was found to be only marginally active (80% inhibition of tumor growth) relative to many other compounds reported previously (6) (Table I). Several of these compounds, such as 3-chloromethylthiochromone-1,1-dioxide (V) (highly active in the test, giving 100% inhibition), could conceivably give rise to compound III *in vivo* by bioreduction and hydrogen chloride elimination (1, 3). The low activity observed with compound III may result from *in vivo* dimerization or other instability such as participation in Michael type reactions. The dimer (IV) was completely inactive in the antitumor test (Table I).

REFERENCES

(1) H. W. Moore, *Science*, **197**, 527 (1977).

(2) S. Omura, H. Tanaka, Y. Okada, and H. Marumo, *Chem. Commun.*, **1976**, 320.

(3) A. J. Lin, R. S. Pardini, L. A. Cosby, B. J. Lillis, C. W. Shansky, and A. C. Sartorelli, *J. Med. Chem.*, **16**, 1268 (1973).

(4) I. H. Hall, K. H. Lee, E. C. Mar, and C. O. Starnes, *ibid.*, **20**, 333 (1977).

(5) I. H. Hall, K. H. Lee, C. O. Starnes, S. A. El Gebaly, T. Ibuka, Y. S. Wu, T. Kimura, and M. Haruna, *J. Pharm. Sci.*, **67**, 1235 (1978).

(6) M. H. Holshouser, L. J. Loeffler, and I. H. Hall, *J. Med. Chem.*, **24**, 853 (1981).

(7) W. M. Welch, C. A. Harbert, R. Sarges, W. P. Stratten, and A. Weissman, *ibid.*, **20**, 699 (1977).

(8) T. L. Gras, *Tetrahedron Lett.*, **32**, 2955 (1978).

(9) C. Piantadosi, C. S. Kim, and J. L. Irvin, *J. Pharm. Sci.*, **58**, 921 (1969).

(10) E. Zeigler, *Monatsh. Chem.*, **18**, 334 (1947).

(11) A. Eschenmoser, *Angew. Chem. Int. Ed. Engl.*, **10**, 330 (1971).

(12) I. Paterson and I. Fleming, *Tetrahedron Lett.*, **33**, 993 (1979).

ACKNOWLEDGMENTS

The work was supported in part by a grant from the Research Council of the University of North Carolina at Chapel Hill.

During the course of this work, M. H. Holshouser was a Henry S. Wellcome Fellow, supported by the American Foundation for Pharmaceutical Education.

The authors wish to thank Dr. Iris H. Hall for her assistance in biological testing performed during this work.

Diazoketone and Chloromethylketone Analogs of Methotrexate as Potential Antitumor Agents

ALEEM GANGJEE *, THOMAS I. KALMAN, and THOMAS J. BARDOS ˣ

Received June 25, 1981, from the *Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14260.* Accepted for publication September 24, 1981. *Present address: Department of Pharmaceutical Chemistry and Pharmaceutics, School of Pharmacy, Duquesne University, Pittsburgh, PA 15282.

Abstract □ The synthesis of 4-amino-4-deoxy-N^{10}-methylpteroyl-(6-diazo-5-oxo)-L-norleucine and 4-amino-4-deoxy-N^{10}-methylpteroyl-(6-chloro-5-oxo)-L-norleucine, analogs of methotrexate in which the γ-carboxyl group is replaced by a diazoketone and a chloromethylketone, respectively, was carried out. The analogs inhibited the growth of leukemia L-1210 cells in culture by 50% at 4×10^{-7} *M* and 2×10^{-7} *M*, respectively, and were effective inhibitors of the synthesis of thymidylate in L-1210 cells *in vitro* ($I_{50} = 3 \times 10^{-6}$ *M*), exhibiting significant antifolate activity. The results demonstrated the feasibility of introducing chemically reactive groups at the γ-position of pteroyl glutamates with retention of biological activity. However, in the systems investigated thus far, there was no evidence of covalent bond formation due to these reactive groups at the active sites of the enzymes.

Keyphrases □ Methotrexate—diazoketone and chloromethylketone as potential antitumor agents □ Antitumor agents—potential, diazoketone and chloromethylketone analogs of methotrexate □ Analogs—diazoketone and chloromethylketone, of methotrexate, potential antitumor agents

Many structural analogs of the clinically useful antitumor agent methotrexate (4-amino-4-deoxy-N^{10}-methylpteroyl-L-glutamic acid, amethopterin) (1), modified at the carboxyl groups of its glutamic acid moiety, have been prepared in the past in attempts to alter the membrane transport properties and to improve the tissue distribution and selectivity of the drug, as well as to circumvent drug resistance. This class of methotrexate derivatives includes a variety of α- and γ-monoesters (2), diesters (3), amides (4), and peptides (4, 5) and also analogs in which the carboxyl groups are replaced by hydrogen (6–8), hydroxymethyl, or methyl (9) groups. As was observed for methotrexate analogs modified at other parts of the molecule (8, 10), with the possible exception of 10-deazaaminopterin

0022-3549/82/0600-0717$01.00/0