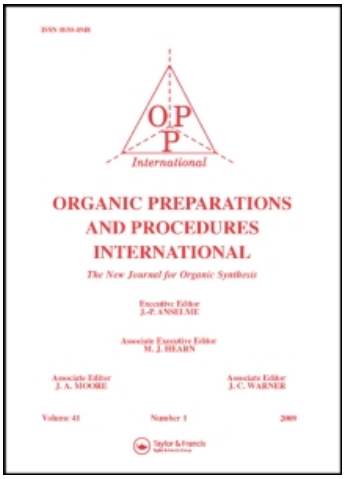

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A PREPARATIVE SCALE SYNTHESIS AND CHROMATOGRAPHIC SEPARATION OF METHOTREXATE-α AND γ-MONOBUTYL ESTERS

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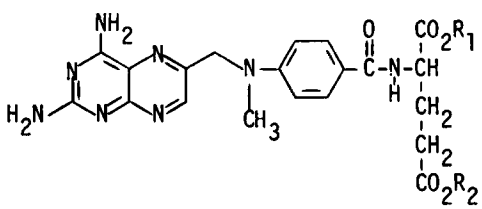
Recently, we wished to synthesize several grams of the methotrexate (MTX)-α and γ-monobutyl esters, 1 and 2, for further evaluation of their anti-cancer activities in higher nonrodent mammalian species. One of us has previously reported several small scale syntheses of 1 and 2,[1] of which one method involved the controlled saponification of MTX-dibutyl ester 3[2] in methanol to yield, after chromatography, only MTX-γ-monobutyl ester 2. Upon scale-up however, this procedure yielded a complex mixture of compounds from which only the MTX-monomethyl esters, 4 and 5, could be isolated.



1 $R_1 = (CH_2)_3CH_3$; $R_2 = H$
2 $R_1 = H$; $R_2 = (CH_2)_3CH_3$
3 $R_1 = R_2 = (CH_2)_3CH_3$
4 $R_1 = CH_3$; $R_2 = H$
5 $R_1 = H$; $R_2 = CH_3$
6 $R_1 = R_2 = H$

We now report that this difficulty may be avoided by the use of DMF or DMSO as the reaction solvent and a slight excess of one equivalent of NaOH as the base. Furthermore, both monobutyl esters 1 and 2 were produced

in the saponification reaction, with the γ-monobutyl ester 2 being the major product. The rate of saponification of diester 3 to monoesters 1 and 2 was much faster in DMSO than in DMF; the reaction being complete in DMSO within four hours after addition of the base. The solubility of esters 1, 2 and 3 in DMSO was sufficient to permit a large scale reaction.

In addition, we have developed two methods for the rapid preparative-scale chromatographic separation of a mixture of 1, 2, and 3 on silica gel utilizing a solution of $CHCl_3/CH_3OH/H_2O$ (40:9:1) as the mobile phase. The first method, which employed dry column chromatography, was convenient for the separation of 0.4 to 2.0 g of the ester mixture. The second method, which utilized a preparative scale HPLC machine, was most useful for the rapid separation of 10 to 16 g of the ester mixture.

Methotrexate monobutyl ester mixture (1 and 2) - To a stirred solution of methotrexate dibutyl ester[2] (77.1 g; 136.1 mmoles) in 600 mL of DMSO at RT was added 139 mL of 1N NaOH (ca. 139.0 mmoles) over a period of 10 min. The reaction temperature rose to 40° and then gradually decreased to ambient temperature over a period of 4 hrs. The solvent was then removed at 40°/1.5 mm pressure to yield a brown colored, viscous oily residue. To this residue was added 1.0 L of cold 1N HCl and the mixture stirred for 1 hr. The resultant solid was isolated by suction filtration, washed well with cold H_2O and then acetone, dried and then ground to a fine powder (54.8 g, 79%). TLC comparison of this powder with authentic samples on silica gel plates[3] with a solution of $CHCl_3/CH_3OH/H_2O$ (40:9:1) as the mobile phase gave R_f values of 0.03-0.04 for methotrexate γ-monobutyl ester 2 and 0.10-0.11 for methotrexate α-monobutyl ester 1 and 0.5-0.52 for methotrexate dibutyl ester 3. TLC comparison of this powder on reversed phase TLC plates[4] using the same mobile phase gave R_f values of 0.41 for 2, 0.70 for 1, and 0.84 for methotrexate dibutyl ester 3. Methotrexate was not present in this butyl ester mixture by TLC analysis. Methotrexate γ-monobutyl ester 2 predominated in

this mixture by qualitative TLC analysis.

Preparative separation of methotrexate α and γ-monobutyl esters

Method A. - A 0.425 g portion of the crude product was disolved in 30 mL of $CHCl_3/CH_3OH/H_2O$ (40:9:1). To this solution was added 11.0 g of dry column grade silica gel,[5] the mixture swirled, and then the volatile liquids were evaporated under reduced pressure to yield a free-flowing yellow finely-divided solid. This was packed on top of a 100 g (3 x 20 cm) silica gel column which was then eluted with the above $CHCl_3/CH_3OH/H_2O$ mobile phase. The first six fractions (20 mL) afforded, after combination and evaporation, approximately 25 mL of methotrexate dibutyl ester 3; further elution (8 x 20 mL fractions) gave 105 mg of 1, then (6 x 20 mL) 25 mg of a mixture of 1 and 2, and then (15 x 20 mL) 243 mg of 2. The mass recovery after chromatography was 94%.

Method B. - Approximately 10-16 g of the crude butyl ester mixture was suspended in 750 mL of warm $CHCl_3/CH_3OH/H_2O$) (40:9:1) solution and then let cool to ambient temperature. Dry column grade silica gel (200 g) was added to the swirled mixture, the solvent was removed under reduced pressure, and the yellow solid residue dried under high vacuum and then ground to a fine powder. This powder was packed into an empty silica gel cartridge, tamped down, and approximately 125 g more fresh silica gel was added to the cartridge, tamped down and the cartridge reassembled.[6] The cartridge was placed in a Waters Associates Prep 500 HPLC[7] and a fresh silica gel cartridge (Waters Associates, Milford, Massachusetts) placed in series after the first cartridge. The mobile phase $CHCl_3/CH_3OH/H_2O$ (40:9:1) was then pumped through both silica gel cartridges at a rate of 250 mL min. In a typical run (∿10 g of crude butyl ester mixture) the first 900 mL of eluate contained 1.2 g of methotrexate dibutyl ester; the following 500 mL of eluate (100 mL fractions) contained 1.7 g of 1,[8] the next 100 mL fraction contained 0.2 g of a mixture of 1 and 2; and the following 15.0 L of eluate (500 mL fractions collected

TABLE. Methotrexate Butyl Esters NMR Spectra

Carbon	R=α Bu	R=γ Bu	R=di-Bu	Ⓐ	Ⓑ
2	162.6	162.5	162.6		162.8
4	162.4	162.2	162.6		162.3
4a	120.8	121.2	121.3		120.7
6	146.3	146.2	145.9		148.8
7	149.1	148.9	149.0		149.1
8a	154.5	154.3	155.0		154.6
9	54.8	54.7	54.8		62.5
10-Me	38.9	38.9	38.9	29.3	
11	150.8	150.6	150.8	152.4	
12	111.0	110.9	110.9	110.4	
13	128.8	128.6	128.8	129.1	
14	121.3	121.2	120.8	120.3	
15	166.4	165.8	166.4	166.8	
17	52.2	52.5	51.8	51.9	
18	25.9	26.7	25.8	26.0	
19	30.7	30.2	30.0	30.3	
COOH	174.6	174.7	172.0		
COOR	172.2	172.5	172.1	(172.3) (172.2)	
OCH_2	63.9	63.3	$(63.4)_\gamma$ $(63.9)_\alpha$	$(60.4)_\alpha$ $(59.9)_\gamma$	
CH_2	30.0	29.9	30.0	-	
CH_2	18.3	18.3	(18.3) (18.4)	-	
CH_3	13.2	13.2	13.2	14.1	

Proton	R=α Bu	R=γ Bu	R=di-Bu	R=H Free Acid
$(C_2)NH_2$	~7.6	~7.6	~7.8	~7.8
$(C_4)NH_2$	~6.6	~6.7	~6.8	~7.2
H_7	8.60 s	8.58 s	8.60 s	8.58 s
$(C_9)CH_2$	4.80 s	4.79 s	4.82 s	4.80 s
10-Me	3.21 s	3.20 s	3.25 s	3.20 s
H_{12}	6.84 d	6.85 d	6.84 d	6.78 d
H_{13}	7.76 d	7.72 d	7.76 d	7.74 d
$(C_{16})NH$	8.45 d	7.98 d	8.30 d	8.14 d
H_{17}	4.41 m	4.32 m	4.46 m	4.38 m
$(C_{18})CH_2$	2.08 m	2.07 m	2.10 m	2.06 m
$(C_{19})CH_2$	2.35 m	2.30 m	2.42 m	2.30 m
COOH	-	-	-	-
OCH_2	4.05 t	3.94 t	$(4.04)_\gamma$ $(4.08)_\alpha$	-
CH_2	1.45 m	1.40 m	1.40 m	-
CH_2				
CH_3	0.83 t	0.81 t	0.87 t	-

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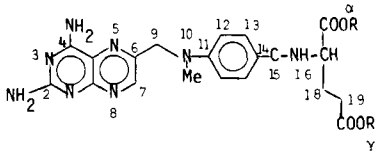
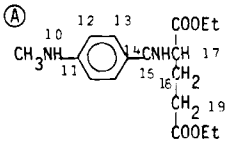


s=singlet; d=doublet; t=triplet; m=multiplet

Ⓐ



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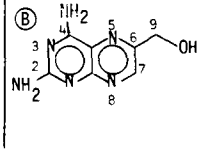

at a flow rate of 500 mL/min) contained 5.7 g of 2.[8] The mass recovery after chromatography was approximately 88%.

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1. A. Rosowsky, G. P. Beardsley, W. D. Ensminger, H. Lazarus and C.-S. Yu, J. Med. Chem., 21, 380 (1978).

2. A. Rosowsky, ibid, 16, 1190 (1973).

3. Silica Gel GF, 250u, 5 x 20 cm, Anatech, Inc., Newark, Delaware.

4. Silica Gel C-18, 200-u, 5 x 20 cm, Whatman, Inc., Clifton, New Jersey.

5. Silica Woelm TSC, ICN Nutritional Biochemicals, Cleveland Ohio.

6. The stainless steel circular frit at one end of a used silica gel cartridge was removed by pushing down on the outer edge of the frit with a small wooden rod, then inserting a spatula under the now elevated opposite edge of the frit, and then gently prying it out of the cylinder. The contents of the cartridge were removed by cautiously shaking out the contained silica gel. The fresh silica gel was then added in portions and tamped down in the usual procedure for packing a dry column. The frit was then replaced with slight uniform thumb pressure at the outer edge.

7. The preparative scale HPLC unit is available from Waters Associates, Milford, Massachusetts.

8. The C-13 and proton magnetic resonance spectra of MTX-monobutyl esters 1 and 2 were identical to those of authentic samples. These data are shown in the table.

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