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TOTAL SYNTHESIS OF (±)-SARKOMYCIN

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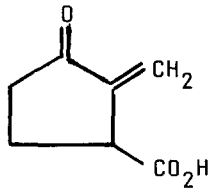
Abstract - The total synthesis of the anticancer agent (±)--sarkomycin **1** is described. Sarkomycin **1** was prepared from 2-phenylthiomethyl-cyclopent-2-en-1-one **5** in four steps in 13% yield. The key reaction was the 1,4-addition of nitromethane to the cyclopentenone **5**.

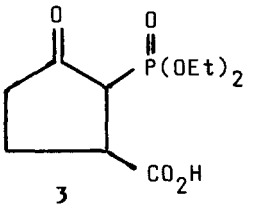
The biological importance and great structural diversity of cyclopentanoid natural products have made these compounds important synthetic targets[1]. Among them, sarkomycin **1** has recently attracted considerable attention. It is produced by a strain of the soil microorganism S.erythrochromogenes, Shygelle species and S.neyagawaensis. Sarkomycin was first isolated by Umezawa et al.[2] in 1953 and its structure established in 1955[3]. In addition to antibacterial and antiphage properties it also shows antitumor activity[4]. Sarkomycin has inhibitory effects on Ehrlich ascites tumors in mice, Yoshida sarcoma, Sarcoma-180 and Hela human carcinoma cell lines. However, it is not active against solid tumors due to the presence of either the carboxylic group or the overall polarity of the molecule **1**, which prevents penetration through the lipid barriers of solid tumors. In spite of the instability of sarkomycin and some problems in its storage, the pharmacological studies of this antibiotic led to merketing in USA of a preparation containing **1** as an antitumor drug.

Due to the wide spectrum of biological activity mentioned above, many synthetic methods directed towards the synthesis of racemic[5] and chiral[6] sarkomycin have been elaborated in numerous laboratories. However, most of these methods employ rather hardly accesible chemicals and complicated experimental procedures.

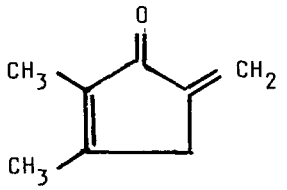
Chart I



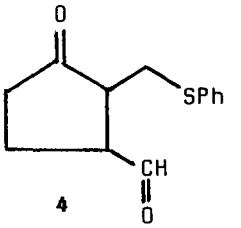
Sarkomycin-**1**



3



Methylenomycin-**2**



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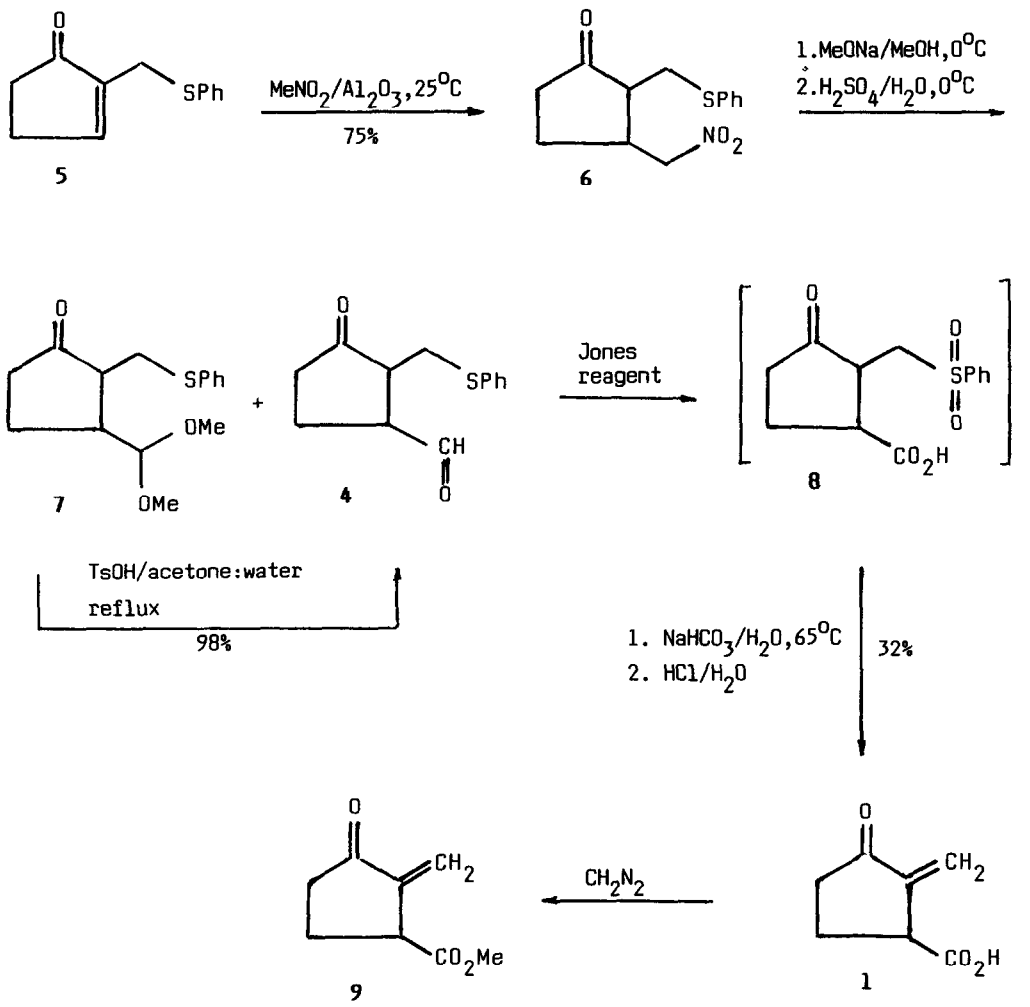
Our interest in sarkomycin **1** as a synthetic target is connected with our general program directed toward the synthesis of functionalized cyclopentenones and cyclopentanones using organic phosphorus and sulfur reagents[7,8]. Particular attention in this laboratory[9,10] was paid to the total synthesis of methylenomycin B **2**, a closely related cyclopentanoid antibiotic. Recently, we have developed a new synthetic approach to sarkomycin **1** in which a key intermediate was 2-diethoxyphosphoryl-3-carboxy-cyclopentanone **3**. Introduction of the exocyclic methylene moiety was effected by means of the Horner-Wittig reaction of **3** with formaldehyde under mild conditions. In the present paper we wish to record the details of our second approach to sarkomycin **1** using easily available reagents and very simple procedures. In this approach a ketoaldehyde **4** is a precursor of **1**.

The synthesis of sarkomycin **1** shown in Scheme I begins with 2-phenylthiomethyl-cyclopent-2-en-1-one **5**[5q] which is readily available in a one-pot reaction from cyclopent-2-en-1-one, 37% aqueous formaldehyde solution and thiophenol in the presence of triethylamine in ethanol as a solvent. In our hands the cyclopentenone **5** was found to be a solid with a melting point 18-20°C. Treatment of cyclopentenone **5** with nitromethane in the presence of aluminium oxide without solvent affords the 1,4-addition product **6** in 63-75% yield. Potassium tert-butoxide in nitromethane was found to be less efficient in this reaction[11].

The structure of the resulting nitro-compound **6** was confirmed by spectroscopic methods. The assignment of the carbon resonances was done with a DEPT 135 experiment which showed four methylene carbons at δ_{13C}=25.7, 33.6, 37.6 and 41.0 ppm as well as two methine carbons at δ_{13C}=51.9 and 79.5 ppm.

Scheme I. Synthesis of (±)-Sarkomycin 1



5 6 7 4 8 9 1

1-D and 2-D-NMR techniques allowed us to assign all the proton resonances and revealed that the signal of the α-ring methine proton lying at δ=2.15--2.32 ppm is overshadowed by a part of a $CH_2C(O)$-multiplet. This was confirmed by the lack of changes in the multiplicity of the CH_2SPh signals upon irradiation of the multiplet at 2.40-2.52 ppm where $CH_2C(O)$ protons are also resonating. The ^{1}H-NMR spectrum revealed also that the addition product **6** consists of the trans and cis isomers in a 9-12:1 ratio. The trans configuration of the major isomer was confirmed by the lack of 1-D and 2-D-NOE

effects between both the exo-methylene groups and methine protons. Our attempts to isolate the minor isomer of **6** and to confirm univocally its cis configuration failed.

In the next step, the classical solvolytic Nef reaction[12] of **6** was carried out in order to convert the nitromethylene moiety into an aldehyde group. However, treatment of **6** with sodium methoxide in methanol and then with cold dilute sulfuric acid gave a mixture of the desired aldehyde **4** (as a 14-16:1 mixture of diastereomers based on the integration of the aldehyde protons), its dimethyl acetal **7** (as a 8:1 mixture of diastereomers based on the integration of the acetal protons) and the starting material **6** in a 2:2.5:0.8 ratio[13]. This mixture was carefully separated by column chromatography eluting with a gradient of hexane/acetone. Dimethyl acetal **7** was then converted into aldehyde **4** in 85-98% yield upon treatment with p-toluenesulfonic acid in refluxing aqueous acetone. The overall yield of **4** from **6** was 53%.

It should be noted that the Nef reaction of **6** using: MeONa/diethyl ether, t-BuOH, MeONa/t-BuOH gave 5, 15 and 37% of aldehyde **4**, respectively, together with the starting material **6**. It should also be noted that the Nef reaction of **6** under reductive[14], oxidative[15] and solid phase[16] conditions as well as the Meyer reaction[17] completely failed.

To complete the preparation of sarkomycin **1** the key intermediate **4** was subjected to oxidation with Jones reagent (three equivalents) resulting in the formation of the transient carboxylic acid-sulfone **8** which gradually eliminates phenylsulfinic acid during workup. For efficient elimination of the phenylsulfonyl group,the sulfone **8** was heated at 65°C for 10 minutes in an aqueous solution of sodium hydrogen carbonate. After acidification and extraction with ether sarkomycin **1** was isolated by column chromatography on silica gel using chloroform/methanol as eluent. It was additionally converted into sarkomycin methyl ester **9** on treatment with diazomethane. The spectral data of both synthetic products **1** and **9** were in an excellent agreement with those reported in literature[5,6]. The total yield of (±)--sarkomycin **1** from **5** was 13%.

In conclusion, we have described here a relatively short and simple synthesis of sarkomycin based on easily available reagents.

EXPERIMENTAL SECTION

Commercially available chemicals were not purified with the exception of nitromethane that was distilled before use. Silica gel 60 F_{254} plates (Merck) were used for analytical chromatography; silica gel 70-230 mesh and silica gel 230-400 mesh were used for column chromatography under normal

pressure and for flash chromatography, respectively. Proton NMR (300.13 MHz) and carbon NMR (75.47 MHz) spectra were recorded on a Bruker MSL spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained on an LKB mass spectrometer.

2-Phenylthiomethyl-3-nitromethyl-cyclopentan-1-one 6. To a vigrously stirred powder of basic aluminium oxide (Merck, 90 active, 70-230 mesh) (2.04 g) nitromethane (3.97 g, 65.12 mmol) and then 2-phenylthiomethyl-cyclopent-2--en-1-one **5** (9.5 g, 46.56 mmol) were added at 0°C. The reaction mixture was stirred at room temperature for additional 9 h and kept for two days. Ether (400 mL) was added and the resulting suspension was stirred for 10 min, filtered and washed with ether (2x150 mL). The combined ethereal solutions were evaporated to give 7.81 g of crude **6**. The aluminium oxide was agitated with acetone or ethyl acetate (2x100 mL) and filtered. The filtrate was evaporated to afford an additional 0.9 g of crude **6**. The crude product was purified by flash chromatography (l=25 cm, ø=2 cm, n-pentane-ether gradient as eluent) giving 1 g of the unreacted **5** and 8.26 g (75%) of pure 1,4--adduct **6** as a mixture of trans and cis-isomers in a 9-12:1 ratio: n_D^{20}= =1.5795. Trans-**6**: ^{1}H-NMR ($CDCl_3$) δ 1.55-1.71 (m,2H,CH_2),2.15-2.32 (m,2H,<u>CH</u>--CH_2SPh and <u>CH_2</u>C(O), 2.91 (m,1H,<u>CH</u>CH_2NO_2), 3.01 (dd,1H,J_{vic}=7.6 Hz, J_{gem}= =14.0 Hz, <u>CH_2</u>SPh), 3.48 (dd,1H,J_{vic}=3.6 Hz, J_{gem}=14.0 Hz, <u>CH_2</u>SPh), 4.36 (dd,1H,J_{vic}=9.1 Hz,J_{gem}=12.6 Hz, CH_2NO_2), 4.91 (dd,1H,J_{vic}=4.7 Hz, J_{gem}= =12.6 Hz, CH_2NO_2) and 7.19-7.38 (m,5H,Ph); ^{13}C-NMR ($CDCl_3$) δ 25.7 (s,CH_2), 33.6 (s,<u>CH_2</u>C(O), 37.6 (s,<u>CH_2</u>SPh), 41.0 (CH_2NO_2), 51.9 (s,CH), 79.5 (s,<u>CH</u>C(O)) 127.6, 129.9, 130.5, 135.5 (m,Ph) and 215.3 (s,C=O); MS (15eV): m/z (%) 265 (M^+,100), 140(17), 126(16), 123(47), 110(54), 109(41), 86(25), 86(25), 84(40) and 81(16). Cis-**6**: ^{1}H-NMR ($CDCl_3$) δ 4.27 (dd,1H,J_{vic}=10.2, J_{gem}=12.6, CH_2NO_2), 4.68 (dd,1H,J_{vic}=5.3, J_{gem}=12.6, CH_2NO_2), other signals are overshadowed by multiples of trans-**6**; ^{13}C-NMR ($CDCl_3$) δ 23.6 (s,CH_2); 33.5 (s, C(O)CH_2); 37.8 (s,CH_2SPh); 4.12 (s,CH_2NO_2); 51.4 (s,CH); 78.2 (s,C(O)<u>CH</u>); 127.7, 130.4, 130.8, 135.7 (m,C_6H_5) 215.0 (s,C=O).
Anal.Calcd for $C_{13}H_{15}O_3NS$ (265.3): C, 58.85; H, 5.70; S, 12.08.
Found: C, 59.19; H, 5.93; S, 12.15.

Nef Reaction of Nitro-compound <u>6</u>. A solution of sodium methoxide freshly prepared by dissolving sodium (0.208 g, 9.06 mmol) in methanol (30 mL) was added dropwise to a solution of **6** (2.0 g, 7.55 mmol) in methanol (40 mL) at 0°C. After stirring for 30 min, the reaction mixture was added to dilute sulfuric acid (220 mL, H_2SO_4:H_2O. 1:3.5) cooled to 0°C. After stirring for an additional 30 min, the reaction solution was extracted with chloroform (2x200 mL). The acidic layer was saturated with sodium chloride and extracted again with chloroform (2x100 mL). The combined chloroform extracts were

washed with brine (2x100 mL), dried ($MgSO_4$) and evaporated. Column chromatography (n-hexane-acetone gradient as an eluent) of the residue afforded: 0.23 g (12%) of the unreacted **6**, 0.54 g (29%) of acetal **7** and 0.5 g (32%) of aldehyde **4**.

2-Phenylthiomethyl-3-dimethoxymethyl-cyclopentan-1-one 7: n_D^{20}=1,5603; ^{1}H-NMR ($CDCl_3$) δ 1.77-1.82 (m,2H,CH_2), 2.07-2.64 (m,4H,$CH_2C(O)$ and CH-CH), 3.18-3.31 (m,2H,CH_2SPh), 3.36 (s,3H,CH_3O), 3.40 (s,3H,CH_3O), 4.35 (d,1H, $^3J_{H-H}$=5.2 Hz, $\underline{CH}(OMe)_2$) and 7.12-7.38 (m,5H,Ph); MS (15 eV): m/z (%) 280 (M^+,20), 204(100), 171(27), 139(55), 110(33), 73(31) and 67(28).
Anal.Calcd for $C_{15}H_{20}O_3S$ (280.38): C, 64.26; H, 7.19.
Found: C, 64.17; H, 6.94.

2-Phenylthiomethyl-3-formyl-cyclopentan-1-one 4: n_D^{20}=1.5710; ^{1}H-NMR ($CDCl_3$) 1.88-2.02 (m,2H,CH_2), 2.16-2.50 (m,2H,$CH_2C(O)$), 2.75-2.80 (m,1H,CH), 2.94 (dd,1H,J_{vic}=8.4 Hz, J_{gem}=13.8 Hz, CH_2SPh), 3.07-3.18 (m,1H,CH), 3.47 (dd, 1H,J_{vic}=4.0 Hz, J_{gem}=13.8 Hz, CH_2SPh), 7.17-7.37 (m,5H,Ph) and 9.73 (d,1H, $^3J_{H-H}$=2.1 Hz, CH(O)); MS (15 eV): m/z (%) 234 (M^+,48), 206(19), 125(17), 124(15), 123(36), 110(57), 97(100), 83(32), 81(19), 79(19), 69(39), 67(15), 55(34), 45(22) and 41(28).

Anal.Calcd for $C_{13}H_{14}O_2S$ (234.32); C, 66.64; H, 6.02.
Found: C, 66.48; H, 6.14.

Hydrolysis of Acetal 7: A solution of **7** (0.282 g, 1 mmol) in aqueous acetone (15 mL, acetone: water 1.5:1) containing p-toluenesulfonic acid monohydrate (0.190 g, 1 mmol) was gently refluxed for 1 h. After removal of the bulk of organic solvents, the residue was treated with acetone (9 mL) and the reaction mixture was refluxed for 1 h. This procedure was repeated once again. Workup consisted of removal of solvent and extraction of the water layer with chloroform (2x10 mL). The water solution was then saturated with sodium chloride and additionally extracted with chloroform (2x10 mL). The combined chloroform solutions were washed with brine (20 mL), dried ($MgSO_4$) and evaporated to give 0.232 g (98%) of analytically pure aldehyde **4**.

(±)-Sarkomycin 1 and Its Methyl Ester 9. To a solution of aldehyde **4** (0.232 g, 0.99 mmol) in acetone (25 mL) was added dropwise at 0°C 1.1 mL (2.97 mmol) of Jones reagent (prepared from 2.0 g of chromium trioxide, 1.65 mL of conc.sulfuric acid diluted with water to a volume of 7.5 mL). After stirring for 3 h at 10-15°C, acetone was evaporated and water (20 mL) was added. The water layer was saturated with sodium chloride and extracted with ether (3x40 ml). The ethereal extracts were washed with brine (2x10 mL)

and evaporated to give the residue, which was dissolved in water (20 mL). To this solution sodium hydrogen carbonate (0.133 g, 1.58 mmol) was added and the resulting mixture was heated at 65°C for 10 min. After cooling to room temperature, the reaction solution was acidified to pH 2 with hydrochloric acid, saturated with sodium chloride and extracted with ether (3x15 mL). The organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Column chromatography on silica gel (chloroform:methanol 100:1 as eluent) of the crude product gave 0.0445 g (32%) of (±)-sarkomycin **1**: ^{1}H-NMR ($CDCl_3$) 2.0-2.68 (m,4H,CH_2CH_2),3.47-3.89 (m,1H,CH), 5.74 (d,1H,J_{gem}= =2.5 Hz, CH_2=) and 6.27 (d,1H,J_{gem}=2.5 Hz, CH_2=); MS (70 eV): m/z (%) 140(M^+,47). The spectral data were identical with those reported in the literature[5n].

Treatment of (±)-**1** with diazomethane in ether at 0°C gave quantitatively (±)-sarkomycin methyl ester **9**: ^{1}H-NMR ($CDCl_3$) 2.10-2.60 (m,4H,CH_2CH_2), 3,60-3.76 (m,1H,CH), 3.81 (s,3H,OCH_3), 5.60 (d,1H, J_{gem}=2.7 Hz, CH_2=) and 6.18 (d,1H, J_{gem}=2.7 Hz, CH_2=), MS (70 eV): m/z (%) 154 (M^+,1). The spectral data were identical with those reported in the literature[5n].

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