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POTENTIAL ANTIBACTERIAL AGENTS. *PART III.* SYNTHESIS OF 7-OXA-SPIRO [5, 9] PENTADECANE-1, 8, 13 TRIONE: A NOVEL MACROCYCLIC LACTONE

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In an attempt towards synthesis of intermediates **(2a)** 2-(1-cyanoethyl) cyclohexanone, **(2b)**, 2-(1-nitroethyl) cyclohexanone reaction of morpholinomethyl cyclohexanone hydrochloride with CH_3CN (or CH_3NO_2) was undetaken. Instead of the two intermediates namely **2a, 2b** the known compound **3** 1-(1-oxo-2-hydroxycyclohexyl)-2-(2-oxocyclohexyl)-ethane was obtained. Oxidation of this compound and its LAH reduction product **4** 1-(1,2-dihydroxycyclohexyl) -2- (2- hydroxycyclohexyl) ethane with CrO_3/Ac OH furnished a hitherto un-described compound **5**, oxa-spiro [5,9] -pentadecane-1,8,13 trione. Its structure was established using spectroscopic techniques such as IR, HRIMS ^{1}H-NMR, ^{13}C-NMR etc. Antibacterial activities of the three synthesized compounds have been evaluated.

Key words: Antibacterial agent, Macrocyclic lactone, 2-morpholinomethyl cyclohaxanone, 2-oxa-spiro [5,9] pentadecane 1, 8,13 trione.

Introduction

The Mannich reaction, an immunoanalogue of the aldol condensation, has been extensively studied by organic chemists due to the resulting Mannich bases which can be either directly employed or used as intermediates in the preparation of organic compounds, (Husain 1990; Rashmi 1990; Stusain 1990; Tramontinic 1990; Fillion 1991; Shide 1996). The most important application of these compounds is in pharmaceutical chemistry, where they have received special attention as anaesthetics (Levy 1938), analgesics (Faizi 1993), antineoplastics (Bo-Gil 1994) and antibiotics (Khadiga 1987).

Mannich reaction is the condensation of a compound having active hydrogen with formaldehyde and amines, more recently cyclic ketones e.g. cyclohexanone. Mannich bases have been found to possess antibacterial and antifungal activities (Cagniant 1980). Similarly the base morpholine is also an integral part of many physiologically active compounds; morpholine DC-89 derivative has been patented as an antitumor agent (Hiromitan 1992).

Mannich reaction employing nitro alkanes in the synthesis of heterocycles, has a significant place in synthetic chemistry. Therefore keeping in view the importance of morpholine cyclohexanone and Mannich reaction as a helpful tool, it was considered worthwhile to study the alkylation reaction of 2-morpholinomethyl cyclohexanone hydrochloride (compound **1**) with methyl nitrate and aceto nitrile, in aqueous medium. It was an attempt towards the synthesis of intermediates which could be transformed into biologically active compounds.

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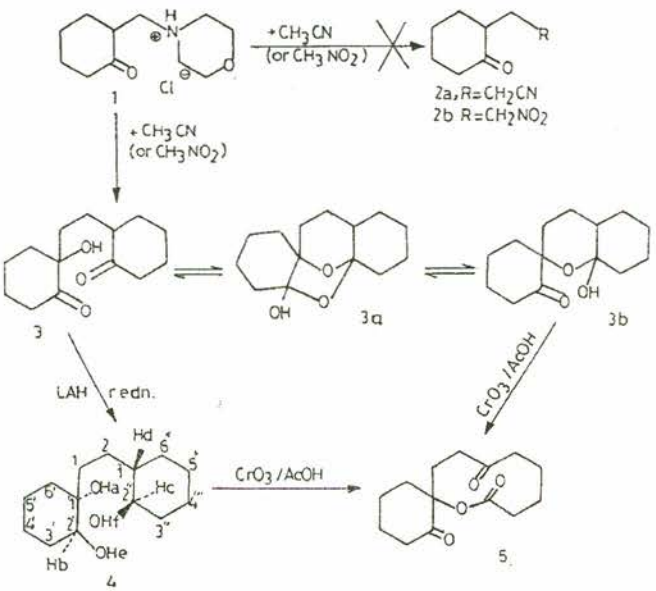
Experimental

Melting points were taken on a Buchi-510 melting point apparatus and are uncorrected. The IR spectra were measured in KBr and $CHCl_3$ on a JASCO A-302 Spectrophotometer. The ^{1}H-NMR spectra were recorded in $CDCl_3$ unless otherwise stated, at 300-500 MHz on Brucker AM-300 ASPECT 3000 Spectrometer. The ^{13}C -NMR spectra were recorded at 75 and 100 MHz on the same instrument. Mass spectra (MS) were determined using a Finnigan Varian MAT 112 or Finnigan MAT 312 double focussing mass spectrometers connected to MAT - 188 data system with PDP 11/34 DFC computer system. Field desorption (FD) measurements were also performed on the MAT-312 spectrometer.

1-(1-oxo-2-hydroxycyclohexyl)-2-(2-oxocyclohexyl) ethane-3. Compound **3** was prepared according to the method of Roth *et al* (196.'). In another route it was obtained by taking compound **1** (2.5g) in different buffer solutions (50ml) separately and the mixture was heated on water-bath for one hour. The sticky solid obtained on cooling was filtered. Crystallisation from ethyl acetate gave white needles of compound **3** m.p. 152°C. (lit m.p. 152°C). The yield in different buffer solutions is given in Table 3. The optimum pH was found to be 5.0.

CrO_3/AcOH oxidation of compound 3 to 5. Compound **3** (1.2g, 0.005 mol) in acetic acid (2.0 ml) was treated with CrO_3 (1.0g, 0.01 mol.) dissolved in acetic acid (3.0 ml) and



Scheme 1.

water (1.5 ml). The mixture was shaken vigorously and then allowed to stand for 18 h at room temperature. The mixture was poured into ice-sodium bicarbonate solution and extracted twice with ether. The combined ethereal layers were then washed with water and dried over anhydrous sodium sulfate. Removal of solvent in *vacuo* and recrystallization from ethyl acetate afforded 7-oxaspiro [5, 9] pentadecane-1, 8, 13-trione compound **5,** as white needles, m.p. 129-130°C (0.44g, 35%). IR max. cm^{-1} 2950-2850 (CH stretching), broad peak 1720-1700 (C=O); EIM m/z, (% intensity) 252 (M^+, 20%) 234 (M^+, 18, 40%), 224 (M^+ 28, 55%), 206 (24%), 195 (40%), 111 (50%), 83 (100%); FDMS m/z 252 (M^+); HRIMS m/z 252. 1371, ($C_{14}H_{20}O_4$ calc. 252. 1361), 234. 1291 ($C_{14}H_{18}O_3$, calc. 234. 1255), 224.1421., ($C_{13}H_{20}O_3$, calc.224. 1412), 206. 1326 ($C_{13}H_{18}O_2$, Calc. 206. 1306), 195. 1008, ($C_{11}H_{15}O_3$, calc. 195. 1021). Analysis; found C, 66.96; H, 8.29% $C_{14}H_{20}O_4$, requires C, 66.65; H, 7.93 & ^{13}C-NMR (100 MHz, $CDCL_3$). Details are given in (Table 1).

*CrO_3/AcOH oxidation of compound 4 to 5.*Compound **4** (0.24g, 0.001 mol) in acetic acid (1.0 ml) was treated with CrO_3 (0.2g, 0.002 mol) dissolved in acetic acid (1.5 ml) and water (0.5 ml). The mixture was shaken vigorously and then allowed to stand for 16 h at room temperature. The mixture was poured into ice-sodium bicarbonate solution and extracted twice with ether. The combined etheral layers were washed with water and dried over anhydrous sodium sulfate. Removal of solvent in *vacuo* and recrystallization from ethyl acetate afforded 7-oxa-spiro [5, 9] pentadecane -1, 8, 13-trione, **5** as white needles, m.p. 129-130°C (60 mg. 25%) identical with the product obtained by the oxidation of compound **4.**

Evaluation of antibacterial activity. Compounds **3, 4** and **5** were tested for their antibacterial activity against both (Gram +ve and -ve) bacteria namely, *Streptococcus lactis Streptococcus pyogenes Staphylococcus aureus, Bacillus subtilis, Bacillus pumilus, Klebsiella pneumoniae, Enterobacter* sp., *Escherichia coli, Pseudomonas* sp, *Salmonella para B* and *Salmonella typhimurium.*

The cultures of bacteria were grown overnight at 37°C and used for testing antibacterial activity. The assay was carried out by overlay agar method (Cruickshank, 1975). The plates were formed with two different media. The thick hard layer or underlay was made with antibiotic assay medium (base agar). The overlay was made with antibiotic assay medium (seed agar). First, the underlay was formed by pouring base agar (melted) plates. When this layer became hard, the seed agar tubes (melted, not too hot to kill the bacteria) were inoculated with organism and poured on sterile hardlay or underlay. The plates were kept at room temperature to get hard. Holes were bored in the medium with the help of sterilized borers. Different dilution of the testing compounds were poured in each hole. The compounds were dissolved in DMF and blank experiment with pure DMF was also performed. Two antibiotics, ampiclox and oxytetracycline were used for comparison. Results of this study have been reported in Table 2 as zones of inhibition after 24 to 48 h of growth at 37°C.

Table 1

^{13}C-NMR spectral data for compounds **3, 4** and **5**

Carbon number	Compounds		
	3	4	5
C-1	30.7	32.9	37.2
C-2	25.5	20.2	39.0
C-1'	79.3	60.3	85.4
C-2'	214.5	72.9	172.6*
C-3'	39.8	33.6	37.8
C-4'	32.9	25.2	29.4
C-5'	25.5	29.2	28.2
C-6'	32.6	29.9	22.5
C-1"	40.7	45.2	207.3
C-2"	212.9	72.4	210.2*
C-3"	35.8	35.5	40.1
C-4"	23.2	24.5	36.5
C-5"	22.8	24.0	34.4
C-6"	21.1	23.3	37.2

1 Recorded in $CDCl_3$; Chemical shift values in ppm; *Interchangeable.

Results and Discussion

Thus the reaction of compound **1,** with compounds such as CH_3CN or CH_3NO_2 in aqueous medium as well as in buff

Table 2
Comparison of antibacterial activities of compounds **3, 4** and **5** with standard antibiotics

S.No.	Name of the Organism	zones of inhibition (mm) 3	4	5	Ampiclox	Oxytetra-cycline
	Gram + ve bacteria					
1.	*Streptococcus lactis*	18	17	17	-	28
2.	*Streptococcus pyogenes*	24	-	-	28	29
3.	*Staphylococcus aureus*	-	-	16	31	25
4.	*Bacillus subtilis*	19	16	17	30	27
5.	*Bacillus pumilus*	20	18	-	28	26
	Gram -ve bacteria					
1.	*Klebsiella pneumonae*	-	19	-	26	27
2.	*Enterobacter* sp.	14	20	19	28	24
3.	*Escherichia coli*	20	20	19	26	19
4.	*Pseudomonas* sp.	20	18	17	29	19
5.	*Salmonella para* -B	25	19	-	26	23
6.	*Salmonella typhimurium*	23	-	27	27	25

Diameter of well, 18.2 mm; concentration, 10 mg ml^{-1} in DMF; (-), no inhibition.

ers, instead of yielding desired compounds; (**2a,2b**) 2-(1-cyanoethyl) cyclohexanone, 2-(1-nitroethyl) cyclohexanone furnished in both the cases, the known product **3** (Roth 1963) m.p. 152°C (152°C lit. m.p) which exists in tautomeric forms *3a,* and *3b* (scheme-1). In addition the ^{13}C-NMR studies of this compound **3** which was not reported earlier, was also undertaken. The ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) showed the presence of only one methine, ten methylene and three quaternary carbons by difference (Table 1).

LAH Reduction of compound 3 to 4. LAH reduction of compound **3** in dry ether yielded 1-(1, 2-dihydroxy cyclohexyl)-2-(2-hydroxyl cyclohexyl) ethane (compound **4,**) m.p. 112°C (lit m. p. 112°C).

The ^{13}C-NMR spectrum (75 MHz) in DMSO of compound **4** is being reported for the first time. It showed 14 carbon resonance in accordance with the deduced formula from HREIMS. The multiplicities of the signals were determined by the edited DEPT spectra (BB.DEPT) which showed the presence of three methine, ten methylene and therefore, one quaternary carbon by difference from the broad band spectrum. the only quaternary carbon present showed resonance, at δ 60.3 which was assigned to the carbon of tertiary alcohol. Out of the three methine signals, two resonated at δ 72.9 and δ 72.4, its downfield chemical shift being evidence of the presence of hetero atom near them. These two were assigned to the methine carbons of the secondary alcohols, while the third methine carbon resonated at δ 45.2. The remaining ^{13}C-NMR chemical shifts of the methylene carbons

Table 3
Effect of pH on the yield of compound **3** from compound **1**

S.No.	pH of buffer solution	% Yield of compound **3**
1	1.74	31.4
2	4.00	33.4
3	5.00	39.2
4	6.90	33.4
5	7.00	11.7
6	11.30	No reaction

are shown in table 1.

CrO_3/AcOH oxidation of compounds 3 and 4 to compound 5. Fiesers oxidation (CrO_3/AcOH) of compound **3** or **4** yielded a crystalline compound **5** m.p. 129-130°C. Its IR showed a broad band at max. 1720-1700 for (C=O) group and at max. 2950-2850 cm^{-1} for -CH_2 stretching. The El mass spectrum of compound **5** showed molecular ion at m/z 252 which was also confirmed by field desorption mass spectrometry. The molecular formula was deduced from HREIMS, m/z 252.1371 which corresponded to $C_{14}H_{20}O_4$, showing five double bond equivalent in the molecule. Its ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) showed 14 carbon resonance in agreement with molecular formula $C_{14}H_{20}O_4$,deduced from HREIMS. The multiplicities of the signals were obtained by edited DEPT spectra (BB.DEPT) showing 10 methylene and therefore, four quaternary carbons by difference from the broad band spectra. The carbonyl carbons were evident from their chemical shifts resonating at δ 210, δ 207.3 and δ 172.6. The fourth quaternary carbon signal resonating at δ 85.4 was attributed to the carbon bearing an electronegative atom or functional group (Table 1). On the basis of the above spectroscopic data, the oxidation product **5** was formulated as 7 oxa-spiro [5,9] pentadecane 1, 8, 13 - trione (Scheme 1,) which was later confirmed by X-ray diffraction method. Its details have been published as separate communications (Parvez *et al* 1998).

Antibacterial activities of compound **3, 4** and **5** were tested by the overlay agar method (Cruickshank 1975) against *Streptococcus lactis, Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis, Bacillus pumilus* (Gram +ve), *Klebsiella pneumoniae Escherichia coli, Enterobacter* sp *Pseudomonas* sp, *Salmonella para B and Salmonella typhimurium* (Gram -ve). The results showed that these compounds are as active as oxytetracycline against Gram -ve organisms like *Escherichia coli. Pseudomonas sp* and *Salmonella para B.* The result of these studies along with their comparisons with the standard antibiotics have been summarized in Table 2.

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