The order of stability of bivalent metal chelates was $UO_2^{2+} > Cu^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+} > Mg^{2+}$.

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Department of Chemistry, Ramnarain Ruia College, Bombay 400 019,
M. S. MAYADEO.
A. M. CHAUBAL.
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PHARMACOLOGICAL STUDIES ON MACROCYCLIC POLYETHER, 15-CROWN-5

IN view of the current interest and importance of macrocyclic polyethers, it is of interest to explore their pharmacological action. Leong *et al.* have shown[1] that rats exposed to the vapours of ethylene oxide tetramer exhibited variable degrees of anoxia, asthenia, testicular atrophy, tremors, convulsions, moribund conditions and death. Later, the same authors reported that oral administration of the tetramer produced central nervous system (CNS) effects.[2] In this communication we report some of our results on the systematic pharmacological study of one of the crown ethers, 1, 4, 7, 10, 13-pentaoxacyclopentane, abbreviated as 15-crown-5.

The ether 15-crown-5, was prepared by the reaction of diethylene glycol with 1,8-dichloro-3,6-dioxaoctane in presence of NaOH in dioxane.[3] (BP 130°/0·2 Torr). The identification and purity was chekced by ^1H NMR spectrum of this compound. A sharp singlet was observed at 3·66 δ in $CDCl_3$ (TMS standard) which is identical with the reported value.[3] The ether was freely soluble in water. Gross behavioral effects were studied on Swiss albino mice of either sex according to the method described by Turner[4]. When administered at dose levels of 5, 50, 100, 250, 500 and 1 mg/kg. I.P. the crown ether showed profound CNS stimulation at a dose level of 50 mg/kg and above as evidenced by hyperactivity, piloerection and pronounced tremors lasting for 150 to 180 mins. LD_{50} was found to be 850 $\pm$ 25 mg/kg in mice. Pretreatment of the animals with atropine sulphate (1 mg/kg; i.p.), trihexyphenidyl HCl (1 mg/kg; i.p.) and cycrimine HCl (1 mg/kg; i.p.), afforded complete protection against the tremorogenic effect of the crown ether (50 mg/kg). Chlorpromazine HCl. (5 mg/kg; i.p.) and diphenhydramine HCl (10 mg/kg; i.p.) conferred only partial protection against the tremors induced by the crown ether (50 mg/kg), in mice and rats. 15-crown-5 at dose levels of 50 and 100 mg/kg; i.p. significantly shortened the duration of pentobarbitone sleeping time in mice. While the control animals treated with 0·1 ml of normal saline 30 min. before the administration of pentobarbitone sodium (30 mg/kg; i.p.), slept for (mean–SEM) 69·2 $\pm$ 2·5 min. animals pretreated with 15-crown-5, slept only for 35·2 $\pm$ 2·6 and 21·3 $\pm$ 2 min respectively, giving a reduction of 49·1% ($P > 0·001$) and 69·2% ($P > 0·001$).

Mycocardial depression studies were carried out on frogs (*Reva hexadactyla*) weighing 80–100 g. 15-crown-5 produced a direct mycocardial depression in perfused frog's heart *in situ* at a dose level of 5 mg. Its action on the heart was not blocked by atropine (1 mg) and mycocardial stimulant effect of adrenaline (10 mg) was not altered by the crown ether. The ether did not produce any effect by itself on isolated frog's rectus abdominis muscle preparation and the contractile effect of acetylcholine (1 mg/1 ml) was not altered.

Guineapig ileum was isolated by the following procedure. Guineapigs of either sex weighing 300–350 g were killed by stunning at the back of the neck and the ileum dissected free from extraneous tissues. On isolated guineapig ileum 15-Crown-5 (100 μg/ml) produced contraction which was antagonised by chlorpheniramine maleate (250 μg/ml). Administration of the crown ether (0·05 ml of 0·1% solution) into plantar aponourosio resulted in severe oedema and inflammation in rats. The oedema volume measured plethysmographically 5 h after administration of 15-crown-5 was found to be 1·36 $\pm$ 0·12 ml. Diphenylhydramine pretreatment (10 mg/kg) afforded 31·6% inhibition ($P > 0·001$) of the oedema produced by the crown ether. Local instillation of the ether (0·1%) into rabbit's eye produced mild hyperaemia and congestion.

Additional studies on anaesthesised dog's blood pressure revealed that intravenous administration of 15-crown-5 at a dose level of 10 mg/kg produced hypotensive effect (about 30–40 mm Hg) which was antagonized by mepyramine maleate (10 mg/1 kg).

It is interesting to note that the administration of linear tetraethylene glycol does not produce the above-mentioned effects. This suggests that cyclisation of the glycol induce remarkable pharmocological effects. The above findings of the pharmacological effects of 15-crown-5 show that it may have many similarities to oxotremoxine in its actions on CNS and it may be an useful adjunct as a pharmocological tool in the evaluation of antiparkinsonism drugs. The studies

also indicate that the ether can be used as an inflammatory agent in evaluating antiinflammatory drugs by the hind paw oedema technique in rats.

Dept. of Pharmacology and Medicinal Chemistry, Research Centre, Madras Medical College, Madras 600 003,
April 23, 1979.

D. SHANKARANARAYAN.
C. GOPALAKRISHNAN.*
S. K. NAZIMUDEEN.
S. VISWANATHAN.
LALITHA KAMESWARAN.
V. KRISHNAN.**

* P.G. Institute of Basic Medical Science, University of Madras, Taramani, Madras 600 042.

** Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560 012).

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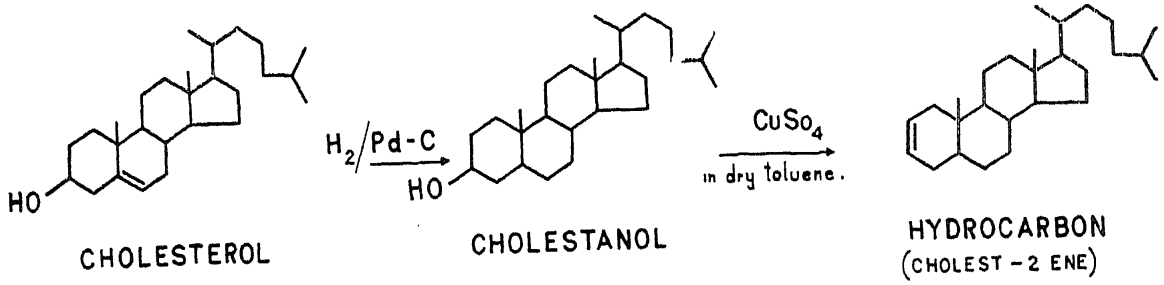
DEHYDRATION OF CHOLESTANOL BY ANHYDROUS-COPPER SULFATE

AN attempt was made to remove the water of crystallisation suspected to be present in cholestanol by refluxing it in dry toluene medium with anhydrous-copper sulfate. But the product of the reaction after chromatographic purification and spectroscopic study was found to be a hydrocarbon (cholest-2 ene) formed by a simple dehydration involving the 3-hydroxylic group of cholestanol.



Although anhydrous copper sulfate is known to be a dehydrating agent (the dehydration of hydroxylated fats like castor oil[1], dehydration of 2,2-dimethyl 3-butanol[2]) yet there is no report regarding the dehydration of a cylic secondary alcohol (like cholestanol) by this dehydrating agent. Generally, cholest-2 ene is obtained from cholestanyl chloride by treatment with quinoline[3]. The present dehydration method is a simple direct and one step process for the preparation of cholest-2 ene from cholestanol. This method can also be applied to other phytostanols like β-sitostanol which give the corresponding 2-ene hydrocarbon.

EXPERIMENTAL

Cholesterol into Cholestanol by Catalytic hydrogenation

About 0·5 gm of cholesterol was hydrogenated using 0·2 g of 10% palladium on carbon catalyst[4] in 50 ml ethyl acetate. After no more absorption of hydrogen the reaction was stopped and catalyst filtered off. The crude cholestanol was crystallised to constant melting point (140–142° C) in ethyl acetate : methanol (7 : 3 v/v) mixture at room temperature.

Dehydration of Cholestanol

About 0·2 g of cholestanol was added to 0·5 g. of anhydrous copper sulfate[5] in 25 ml toluene (dried-over sodium) and the mixture was refluxed under anhydrous conditions for 10 hrs. The product was worked up by filtering off the copper sulfate, washing with 3 × 5 ml of dry toluene and the combined toluene solution evaporated to dryness, under vacuum. The crude product was chromatographed over 10% argentized silicagel (1 : 100) twice, using 10% benzene: petroleum ether (60–80° C) mixture as eluant. Finally the product was crystallised to constant melting point (69–70° C) in ethylacetate : methanol (5 : 1 V/V) mixture, when the hydrocarbon (cholest-2 ene) crystallised out at 5° C as white solid (Yield 33%).

On TLC using 40% argentized silicagel G plate with 10% benzene : petroleum ether mixture as developing solvent, the above hydrocarbon gave a single spot (Rf 0·35). Following are the spectroscopic details for the hydrocarbon:

γ_{max}^{Nujol} cm^{-1} 3000, 2900, 1600, 1480, 820

$\tau_{100\,MHZ}^{CDCl_3}$ 4·4 (2H, olefinic, m) 7·92 (2H, allylic CH_2, m) 8·2 (2H, allylic CH_2, m) 9·08–9·3 (15H, 5 methyls)

m/e (rel. int.) 70 ev 370 (M^+, 83%), 316 (M^+ − 54, 55%)
257 (M^+ − Side chain, 17%)

Found C 87·42, H 12·47%, $C_{27}H_{46}$ requires C 87·57, H 12·43%.

Sandal Research Centre, Bangalore 560 003,
April 2, 1979.

K. H. SHANKARANARAYANA.
K. S. AYYAR.*

* Forest Research Institute and Colleges, Dehra Dun,