The necessity for direct comparison prompted us to prepare the authentic samples by an unequivocal route. The desired compounds were synthesized from the isomeric 2-hydroxyestrone methyl ethers[4] according to the method worked out by Gallagher and his co-workers.[6] 2-Methoxyestrone 3-benzyl ether was transformed into the Δ^{16}-enol acetate with isopropenyl acetate and a catalytic amount of sulfuric acid. Treatment with *m*-chloroperbenzoic acid and cleavage of the epoxyacetate with sulfuric acid gave the 16α-hydroxy-17-ketone in a satisfactory yield. Removal of the benzyl group at C-3 by catalytic hydrogenolysis afforded the required 2-methoxy-16α-hydroxyestrone as colorless needles (from acetone–hexane). mp 201.5—204°. The synthetic sample proved to be identical with metabolite I by mixed melting point, IR, NMR and mass spectral measurements. 2,16α-Dihydroxyestrone 3-methyl ether was also prepared from 2-hydroxyestrone 3-methyl ether by the same reaction sequence as for its positional isomer. The desired compound was obtained in a reasonable yield as colorless prisms (from methanol). mp 195.5—198°. Subsequent acetylation in the usual manner provided the 2,16-diacetate as colorless prisms (from acetone–hexane). mp 219.5—222°. The identity of these synthetic samples and metabolite II and its 2,16-diacetate was justified by the usual criteria, *i.e.* IR, NMR and mass spectra and chromatographic behaviors.

To the best of our knowledge this is the first recorded instance of a bioconversion of estrone to catechol estrogen having the 16,17-ketol structure, though the isolation of 2-methoxyestrone has been reported.[7] It is also to be noted that *in vivo* O-methylation takes place at both hydroxyl groups in ring A to yield isomeric catechol estrogen monomethyl ethers. This finding is in good accord with the recent paper dealing with an *in vivo* conversion to 2-hydroxyestrone 3-methyl ether.[8]

Further studies on the biliary estrogen metabolism are being conducted and the details will be reported in the near future.

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Carboxylation of Cyclohexanone by Carbon Dioxide and Potassium Pyrrolidone in Aprotic Solvents

It has been known that biotin, sometimes called as vitamin H, is concerned with carboxylation and decarboxylation in living bodies.[1] This biological function by biotin has so far attracted the attention from a chemical point of view with regard to the capability of CO_2 donor-acceptor nature of biotin-like compounds.[2] As an extention of previous work,[3]

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which concerned with the carboxylation of cyclohexanone by potassium phenoxide-carbon dioxide complex, we have studied on the reaction in the presence of potassium pyrrolidone and carbon dioxide in dimethylsulfoxide (DMSO), and have found that the carboxylation of cyclohexanone can take place. Experimental procedure and result are as follows.

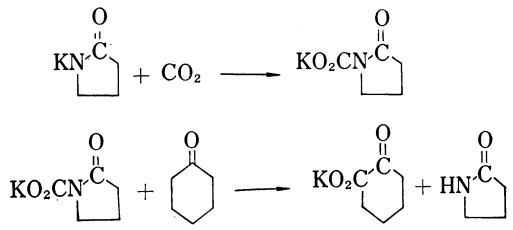
Potassium (8 g) was added to pyrrolidone (30 g) in anhydrous benzene (300 ml). After 1 hour's gentle refluxing the product was filtered off under the atmosphere of nitrogen and excess pyrrolidone was washed with anhydrous benzene. The product thus prepared was snow-white and hygroscopic. The potassium pyrrolidone sample (12.3 g, 0.1 mole) was then added to a given aprotic solvent and gaseous carbon dioxide was bubbled into the reaction system stirred electromagnetically. Then 4.9 g (0.05 mole) of cyclohexanone was introduced into the solution. After several hours' reaction below 80° a portion of reaction mixture was poured into an aqueous solution of hydrochloric acid and was followed by extraction with ether. To the extract was added an ether solution of diazomethane. The methyl ester of carboxylates formed was quantitatively analysed by gas chromatography using naphthalene and anthracene as internal standards. Table I shows the results obtained with various solvents.

Table I. Carboxylation of Cyclohexanone in the Presence of Potassium Pyrrolidone and Carbon Dioxide

Solvent	Temp. (°C)	Reaction time (hr)	Yield (%)	
			Monocarboxylate	Dicarboxylate
DMF	room temp.	2.5	0.3	—
DMF	70—80	4.0	1.1	0.8
DMSO	70—80	4.0	10.3	0.6
N-Methyl pyrrolidone	70—80	4.0	1.2	—
HMPA	70—80	4.0	0.1	—

Potassium pyrrolidone was soluble only with difficulty in the solvent such as dimethylformamide (DMF) or hexamethylphosphoric triamide (HMPA). When DMSO was used as the solvent, the yield of carboxylate was appreciable as shown in Table I. This can probably be ascribed to a higher solubility of DMSO for potassium pyrrolidone.

When carbon dioxide was bubbled into the suspension of potassium pyrrolidone, an exothermic reaction was usually recognized. This would imply that a certain reaction took place between potassium pyrrolidone and carbon dioxide. It is thereby suggested that the carboxylation of cyclohexanone proceeds according to the scheme:



The reaction scheme could be regarded as a biotin model reaction in the sense that the carboxylation proceeds *via* the N-carboxylated intermediate. Further works are in progress.

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