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SHORT COMMUNICATIONS

Studies on the hypolipidemic and estrogenic activities of 2,8-dibenzylcyclooctanone and its analogues

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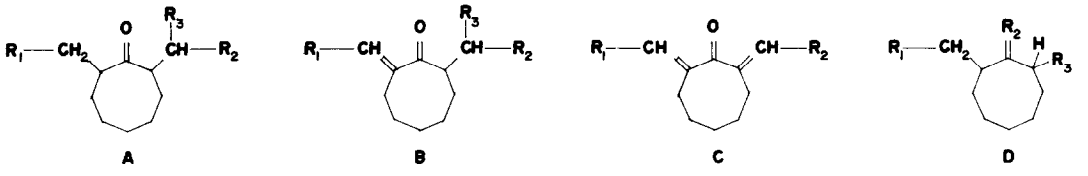
It has been suggested that the hypolipidemic activity of 2,8-dibenzylcyclooctanone (DBCO) in rats is associated with its estrogenicity [1, 2]. In this report, studies are described which corroborate the estrogenic activity of DBCO. In addition, in order to assess whether structural modification could separate the hypolipidemic and estrogenic properties, we have investigated the effects of a series of analogues of DBCO on serum lipids and on estrogenic activity in rats. The results of these studies are described in the present communication.

The compounds were kindly supplied through the courtesy of Dr. C. Piantadosi, University of North Carolina, Chapel Hill. Compounds listed in Table 1 were suspended in 2% Tween-80 and administered at 9:00 a.m. by gavage to male albino Charles River CD rats (ten rats/group weighing 140–160 g) at a dose of 100 μmoles/kg/day for 7 days. In each experiment, DBCO was used as reference compound. On the last day of treatment, food was withdrawn at 8:30 a.m. and decapitation was initiated at 12:00 noon. Food intake was recorded as well as the weights of the adrenals, seminal vesicles and ventral prostate. Serum cholesterol was measured by the method of Zlatkis *et al.* [3] as modified for the Auto-analyzer. Serum phospholipid [4] and triglyceride [5] levels were also determined.

The estrogenicity of orally administered DBCO was assessed in females by means of three tests: the Allen Doisy test in rats [6, 7], the uterotrophic assay in mice [7], and gonadotrophin suppression in parabiotic rats [8]. Although the exact ED_{50} was not determined in the Allen Doisy test, it was found that, after 3 days, 60 per cent of the rats exhibited cornification of the vaginal smear at 2 mg/kg/day, and 100 per cent showed an estrus smear at 3 mg/kg/day. DBCO was tested at six dose levels in the uterotrophic assay and, at a dose of 850 μg/kg/day, caused a 3-fold increase in uterine weight as compared to controls. In addition, 20 mg/kg/day of DBCO for 10 days produced a 100 per cent suppression of gonadotrophins in parabiotic rats; in the same test, the uterine weight of the castrated partner was significantly increased. This

Table 1. Derivatives of DBCO



Compound No.	Type	R_1	R_2	R_3
DBCO	A	C_6H_5	C_6H_5	H
1	A	C_6H_5	4′-ClC_6H_4	H
2	A	C_6H_5	4′-$NH_2C_6H_4 \cdot HCl$	H
3	A	4′-FC_6H_4	4′-FC_6H_4	H
4	A	4′-HOC_6H_4	4′-HOC_6H_4	H
5	A	4′-$CH_3COOC_6H_4$	4′-$CH_3COOC_6H_4$	H
6	A	4′-ClC_6H_4	4′-ClC_6H_4	CH_3O
7	A (*cis*)	C_6H_5	C_6H_5	CH_3O
8	A (*trans*)	C_6H_5	C_6H_5	CH_3O
9	A	2′-$CH_3C_6H_4$	2′-$CH_3C_6H_4$	H
10	A	C_6H_{11}*	C_6H_{11}*	H
11	A	$C_{10}H_7$†	$C_{10}H_7$†	H
12	B	4′-ClC_6H_4	C_6H_5	H
13	B	C_6H_5	C_6H_5	CH_3O
14	B	4′-ClC_6H_4	4′ClC_6H_4	CH_3O
15	C (*cis, trans*)	C_6H_5	C_6H_5	
16	C (*trans, trans*)	C_6H_5	C_6H_5	
17	C	4′-$NO_2C_6H_4$	4′-$NO_2C_6H_4$	
18	D	C_6H_5	O	H
19	D	4′-ClC_6H_4	H,OH	4′-$ClC_6H_4CH(OCH_3)$
20	D	C_6H_5	H_2	$C_6H_5CH_2$

* C_6H_{11} = cyclohexyl.
† $C_{10}H_7$ = 1-naphthyl.

Table 2. Effect of structural analogues of DBCO on serum lipids and on weights of adrenals and seminal vesicles in male rats receiving 100 μmoles/kg/day p.o. for 7 days

Compound No.	Dose (mg/kg/day)	Per cent change from control*				
		Serum lipids			Tissue weight	
		Cholesterol	Phospholipids	Triglycerides	Adrenals	Seminal vesicles
DBCO†	31	−79‡	−53‡	−49‡	+105‡	−54‡
1	34	−53‡	−23‖	NS	+35	−50‡
2	36	−71‡	−40‡	−40§	+76‡	−61‡
3	34	−28‡	−15‡	NS	+15	•
4	34	NS	NS	NS	+17	NS
5	42	NS	NS	NS	NS	NS
6	40	NS	NS	NS	NS	NS
7	33	NS	NS	NS	NS	+23
8	33	−58‡	−25‡	+58‡	+32‡	−27‡
9	33	−51‡	−26‡	NS	+41‡	−45§
10	33	−38‡	−13§	NS	NS	−35
11	40	NS	NS	NS	NS	−21
12	34	NS	NS	NS	NS	NS
13	33	NS	NS	NS	NS	NS
14	40	NS	NS	NS	NS	NS
15	30	−17	−12	−25	+22	NS
16	30	−22‡	−14	NS	NS	−25
17	39	NS	NS	NS	NS	NS
18	22	+17	NS	NS	NS	NS
19	41	NS	NS	NS	NS	NS
20	29	NS	−10	NS	−14	NS

* Serum lipids were calculated as mg/dl and tissue weight as mg/100 g of body weight.
† Average of seven experiments.
‡ $P < 0.001$.
§ $P < 0.01$.
‖ $P < 0.05$.
• Data inconclusive.

finding indicates that the gonadotrophin suppression was due to the estrogenic activity of the compound [9]. Thus, although the compound is a very weak estrogen, estrogenic activity was found at doses which were hypolipidemic [2].

The effects of the structural analogues of DBCO on serum lipids and on the weights of adrenals and seminal vesicles are summarized in Table 2. The changes observed in the weights of the ventral prostate were similar to those obtained with the seminal vesicles and are not included in the table. The data are expressed as percentage change from untreated animals used as control; only values which were significantly different from controls are presented. DBCO was the most potent hypolipidemic and estrogenic compound in the series. Only compounds containing the dibenzylcyclooctanone system were active. Some hypolipidemic activity was retained when the aromatic nucleus was substituted by a cyclohexane ring, e.g. as in compound 10. Cholesterol-lowering activity was observed with the *p*-chloro (compound 1), *p*-amino (compound 2), *p*-fluoro (compound 3) and *o*-methyl (compound 9) derivatives, as well as with the α-methoxy analogue (compound 8) of DBCO. No activity was detected in compounds containing a benzylidene group or when the ketone group was reduced. Triglyceride-lowering activity was found only with compounds 2 and 15.

All compounds showing hypocholesterolemic activity, except the weakly active compound 15, also decreased the weights of the seminal vesicles and ventral prostate, and increased adrenal weight. Such changes in the weights of these organs are known to occur in male rats treated with estrogenic compounds. Compound 3, which in male rats produced inconclusive changes in seminal vesicle weight was tested for estrogenic activity in female rats by the Allen Doisy test. At a dose of 30 mg/kg/day for 3 days, the compound caused vaginal cornification in all animals.

It was concluded that, in this series of structural analogues of DBCO, the hypocholesterolemic activity was correlated with estrogenicity.

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