Mechanism of Prostaglandin Biosynthesis. III. Catecholamines and Serotonin as Coenzymes

Sir:

The biosynthesis of prostaglandin-E_1 (PGE_1) from 8,11,14-eicosatrienoic acid by ovine seminal vesicle preparations was shown to require reduced glutathione (GSH) and hydroquinone (HQ),[1] but the roles of the latter compounds in this oxidative cyclization reaction have not been clearly defined. Accordingly to Nugteren, *et al.*,[2] 2.4 mol of GSH was consumed per mole of fatty acid metabolized and HQ was required as an antioxidant. In this communication, experimental evidence is presented to show that catecholamines and serotonin can act as coenzymes by providing the reducing equivalents for prostaglandin synthesis.

A wide variety of compounds was observed to replace the HQ in this oxidative cyclization reaction.[1] This raises the question as to the identity of the natural coenzyme. As catechol could substitute for HQ, albeit poorly, in the bovine seminal vesicle system (BSVM), we suspected that the natural coenzyme may be the catecholamines. It was found that both L-norepinephrine and L-epinephrine effectively supported prostaglandin formation whereas dopamine and L-3,4-dihydroxyphenylalanine were less effective. More striking, however, is the finding that the ratio of prostaglandins can be altered during biosynthesis. Table I shows

Table I. Effect of Coenzymes on Prostaglandin Synthesis

	μmol			
	PGA[a]	PGD	PGE	PGF
HQ (pH 7.5)	1.3	3.0	11.0	1.0
HQ (pH 9)	2.6	0.7	1.7	
L-Epinephrine (pH 9)	1.4	2.9	13.4	11.5
Serotonin (pH 9)	2.4	6.3[b]	18.3	4.4
5-Hydroxyindole-3-acetic acid (pH 9)		3.8	11.8	2.2

[a] PGA was formed in the work-up. [b] In some experiments, yields of PGD were as high as 10 μmol. The reaction mixture consisted of 65 μmol of arachidonic acid (5 μCi of tritium), 1.64 mmol of coenzyme, and 1 g of BSVM in a total volume of 100 ml of 0.05 *M* Tris buffer at the pH indicated. After incubation for 1 hr at 37°, the contents were acidified to pH 2.0 and extracted with ethyl acetate. The ethyl acetate residue was chromatographed over a silicic acid column and the radioactive peaks were combined and assayed.

that the ratio of 11-dehydro-PGF[3] (PGD), PGE, and PGF using HQ was 3:11:1, respectively, whereas this ratio was changed to 3:13:12 using L-epinephrine. Because a substantial amount of tryptophan was found in the supernatant fraction of bovine seminal vesicles, we examined the hydroxyindoles as possible coenzymes. Both serotonin and 5-hydroxyindole-3-acetic acid (5-OH-IAA) were capable of supporting prostalgandin synthesis. While L-epinephrine enhanced PGF formation, serotonin favored the formation of PGD.

To confirm that the reducing equivalents in prostaglandin formation are derived from these compounds, we selected 5-OH-IAA as a model for stoichiometric studies. When the disappearance of 5-OH-IAA-^{14}C was correlated with the amount of prostaglandin formed, it was noted that approximately 2 mol of 5-OH-IAA was consumed per mole of prostaglandin formed (Table II). When GSH was included

Table II. Stoichiometry of 5-OH-IAA Consumption during Prostaglandin Formation[a]

Additions	μmol of: Total PG formed (A)	μmol of: 5-OH-IAA used (B)	Ratio B/A
5-OH-IAA	1.4	2.9	2.1
5-OH-IAA + GSH	2.4	0.49	0.2

[a] The system contained 3.3 μmol of arachidonic acid (5 μCi of tritium for PG assay), 16.2 μmol of 5-OH-IAA (2 μCi of ^{14}C for 5-OH-IAA assay), 13 μmol of GSH (where indicated), and 100 mg of BSVM in 10 ml of 0.05 *M* Tris buffer (pH 9.0). The mixture was incubated at 37° for 30 min and worked up as in Table I. The prostaglandins were eluted off the column with a gradient system of benzene–ethyl acetate. 5-OH-IAA was eluted from the same column with a 6:4 mixture of ethyl acetate–benzene.

in the incubation mixture, the amount of 5-OH-IAA utilized decreased to a value of 0.2 per mol of prostaglandin formed. This result clearly shows that 5-OH-IAA is the source of reducing equivalents. Furthermore, GSH appears to reduce the oxidized form of 5-OH-IAA back to the reduced form. The effect of GSH was reported to be specific and could not be replaced by other SH reagents.[2] Thus, it is possible that this reduction is enzymic or that GSH also behaves as an allosteric effector.

Catecholamines, serotonin, and prostaglandins are all widely distributed throughout various tissues. One could speculate that catecholamines and serotonin may be the natural coenzymes in regulating the *in vivo* biosynthesis of prostaglandins. Furthermore, it may be that one of the important physiological roles of these hormones is to furnish reducing equivalents for a variety of oxygenation reactions.

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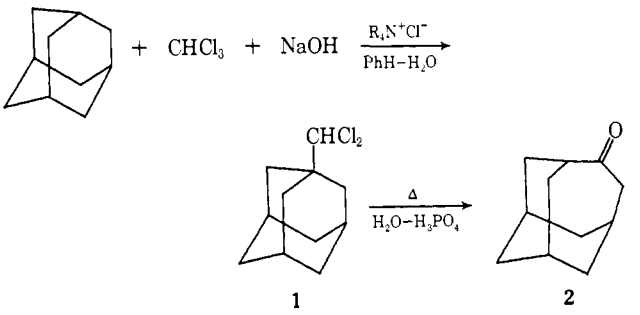
(1) D. H. Nugteren, R. K. Beerthuis, and D. A. Van Dorp, *Recl. Trav. Chim. Pays-Bas*, 85, 405 (1966).
(2) D. H. Nugteren, R. K. Beerthuis, and D. A. Van Dorp, *Prostaglandins, Proc. Nobel Symp., 2nd, 1967*, 2, 45 (1967).
(3) E. Granstrom, W. E. M. Lands, and B. Samuelsson, *J. Biol. Chem.*, 243, 4104 (1968).

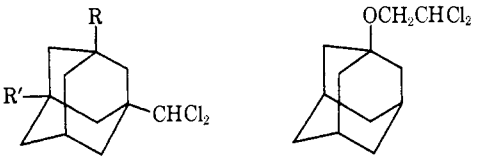
Dichloromethyladamantanes. Formation *via* Carbene Insertion and Hydrolytic Rearrangement to Homoadamantanone

Sir:

We wish to report the rearrangement of 1-dichloromethyladamantane (**1**) to homoadamantanone (**2**) in the presence of hot phosphoric acid. We also wish to report the extraordinarily efficient dichlorocarbene insertion reaction with adamantanes giving 1-dichloromethyladamantane (**1**) exclusively and in nearly quantitative yield. It was the first successful dichlorocarbene insertion into a saturated hydrocarbon.



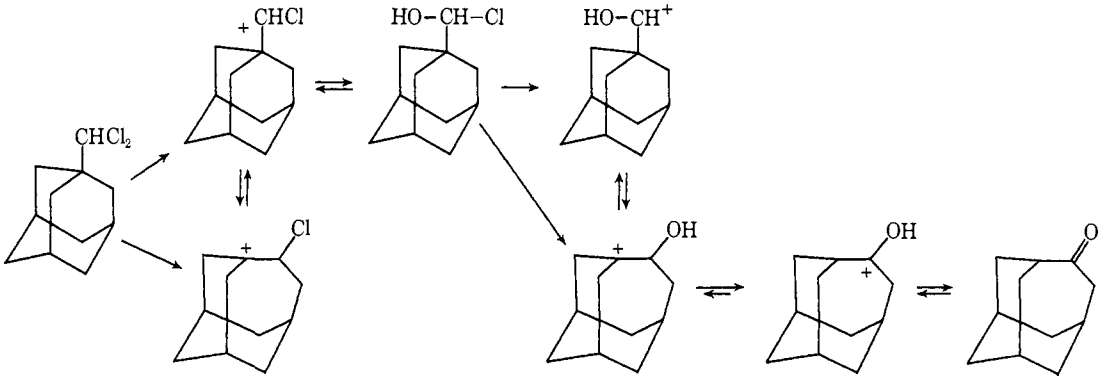
A mixture of 13.6 g of adamantane, 200 ml of 50% (w/w) aqueous sodium hydroxide, 20 ml of benzene, and 0.4 g of triethylbenzylammonium chloride[1] was vigorously stirred to emulsify the mixture at 43°. Into the emulsion was added dropwise 80 ml of chloroform during 6 hr. After the addition was over, the mixture was further stirred for 0.5 hr. Usual work-up gave 54% 1-dichloromethyladamantane (**1**) (91% based on adamantane consumed) and 41% unreacted adamantane: nmr spectrum of **1** (TMS, CCl_4) τ 4.66 (1 H), 7.8–8.15 (3 H), 8.1–8.4 (12 H); mass spectrum *m/e* 220 (relative intensity 20), 219 (17), 218 (32), 217 (23), 149 (69), 147 (38), 139 (40), and 137 (100); ir spectrum 2925, 2900, 1448, 1340, 1215, 1095 938, 810, 730, and 690 cm^{-1}; bp 114–115° (8 mm).



2a, R = CH_3; R' = H
b, R = CH_3; R' = CH_3
c, R = OCH_3; R' = H

3

Chart I



Methyladamantane and dimethyladamantane also gave corresponding dichloromethyl derivatives **2a** and **2b**, respectively, in nearly quantitative yield. 1-Methoxyadamantane afforded **2c** together with an approximately equal amount of **3**, but both in poor yields. 1-Bromoadamantane and adamantane-1-carboxylic acid did not afford corresponding dichloromethyl derivatives in appreciable amounts under similar conditions.

Usually carbene insertion is not a very useful procedure for organic synthesis because of its poor selectivity leading to many isomers and by-products.[2] The present reaction, however, represents the successful preparative application of the carbene insertion reaction. It is noteworthy that enormously high positional selectivity (bridgehead to bridge reactivity ratio, BH/BR) was observed in the present system in remarkable contrast to methylene insertion which showed "moderate" positional selectivity[3] toward open-chain or cyclic alkanes and to nitrene insertion which showed a 7:1 reactivity ratio (BH/BR) toward adamantane[4] (see Table I).

Table I. Selectivity of Carbene Insertion into Polycyclics (BH/BR)

Polycyclic hydrocarbon	Carbene or nitrene	BH/BR	Ref
Adamantane	NCO_2Et	*Ca.* 7	*a*
Adamantane	CCl_2	∞	*b*
1-Methyladamantane	CCl_2	∞	*b*
1,3-Dimethyladamantane	CCl_2	∞	*b*
Bicyclo[2.2.2]octane	CH_2	*Ca.* 1	*c*
	$CHCO_2CH_3$	*Ca.* 1	*c*
	$C(CO_2CH_3)_2$	0.7	*c*

[a] Reference 4. [b] This work. [c] M. R. Willcott, III, Ph.D. Thesis, Yale University, New Haven, Conn.

Another point to note is that only one dichloromethyl was introduced into adamantane by this insertion reaction.[5] The exclusive monosubstitution can be rationalized by the retarding effect of the dichloromethyl substituent against the second insertion reaction. The interpretation was supported by the observation that an electron-withdrawing group ($\sigma_I > 0$) on adamantane was found to markedly retard the insertion as cited above.

The dichlorocarbene insertion is also applicable to cyclic and acyclic hydrocarbons in general (the details will be published soon).

A mixture of 1.09 g of dichloromethyladamantane and 20 ml of 85% phosphoric acid was heated to 110–

(1) The procedure is a modification of Makosza's dichlorocarbene addition: M. Makosza and M. Wawrzyniewicz, *Tetrahedron Lett.*, 4659 (1969).

(2) The insertion reaction of methylene with common solvents is well known, *e.g.*, with ether: H. H. Frey and M. A. Voisey, *Chem. Commun.*, 454 (1966); also see ref 3 and 4.

(3) Relative insertion rates: the secondary/primary ratio was 2:1 for propane and the tertiary/primary ratio was 7:1 for isobutane: R. F. Ring and B. S. Rabinovitch, *Can. J. Chem.*, **46**, 2435 (1968). Singlet CH_2 was reported to be much less discriminative: J. W. Simons, O. J. Mazac, and G. W. Taylor, *J. Phys. Chem.*, 72, 749 (1968); see also H. M. Frey, *J. Amer. Chem. Soc.*, **80**, 5005 (1958), where the corresponding ratios were observed to be 2.6 and 6.1, respectively.

(4) D. S. Breslow, E. I. Edwards, R. Leone, and P. von R. Schleyer, *ibid.*, **90**, 7097 (1968).

(5) Attempted dichlorocarbene insertion with the isolated mixture gave 1-dichloromethyladamantane almost quantitatively without appreciable formation of bis(dichloromethyl)adamantane.

130° for 47 hr under nitrogen. The mixture was poured onto ice water and extracted with benzene. From the benzene extract were obtained homoadamantanone (69% yield), adamantanecarboxyaldehyde (6%), and recovered dichloromethyladamantane (6%). Identification of homoadamantanone[6] was made by means of: ir (2910, 2850, 1695, 1450, 1360, 1280, 1180, 1080, 940, and 800 cm^{-1}); nmr (TMS, CCl_4, τ 7.2–7.57 (1 H), 7.45–7.6 (2 H), 7.7–8.5 (13 H)); and mass spectrum (*m/e* 163 (relative intensity 100), 136 (13), 135 (85), 121 (25)). The melting point of isolated homoadamantanone was 267–268° (uncorrected) without further purification (lit.[6] 270–271.5°). Its oxime melted at 145.5–147.5°. The amount of 1-adamantanecarboxyaldehyde formed was found to increase with temperature, being one of the major products over 170°. At higher temperatures, a small amount of adamantane was also obtained.

Although the actual mechanism of the hydrolytic rearrangement to homoadamantanone is not yet elucidated, one involving an intermediate carbonium ion (Chart I) seems to be plausible by analogy with the well-known adamantylmethyl type.[7]

(6) Homoadamantanone: (a) G. B. Gill and R. M. Black, *J. Chem. Soc. C*, 671 (1970); (b) P. von R. Schleyer, E. Funke, and S. H. Liggero, *J. Amer. Chem. Soc.*, 91, 3965 (1969). (c) Trimethylhomoadamantanone: K. Bott, *Tetrahedron Lett.*, 1747 (1969).

(7) H. Stetter and P. Goebel, *Chem. Ber.*, 96, 550 (1963).

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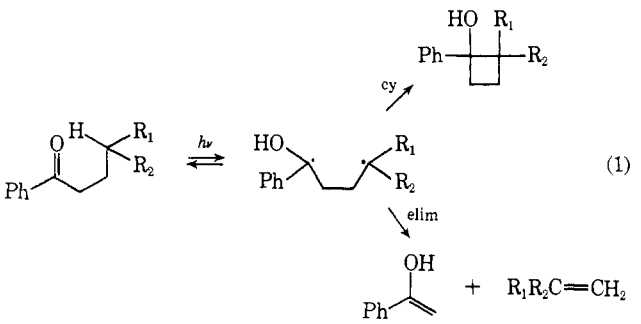
Photochemistry of Methyl-Substituted Butyrophenones. The Nature of the 1,4-Biradical Intermediates[1]

Sir:

The type II photoelimination and cyclization reactions of aryl alkyl ketones having a γ-hydrogen have been the subject of extensive investigations.[2-7] It is generally agreed that a 1,4-biradical intermediate formed upon γ-hydrogen abstraction by the carbonyl excited state gives rise to elimination and cyclization products as well as ground-state ketone formed by biradical disproportionation (eq 1). Whereas the effect of structure and solvent on the rate constant for γ-hydrogen abstraction and quantum yield for photoelimination have been extensively studied, little is known about the nature of the biradical and the factors which govern the relative rate constants for cyclization and elimination as well as the stereochemistry of

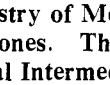
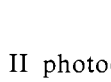
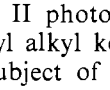
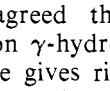
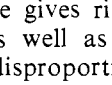
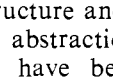
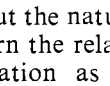
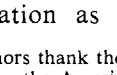
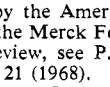


(1)

cyclization. As part of a study designed to provide information about the nature of 1,4-biradical intermediates,[8] the photochemistry of the methyl-substituted butyrophenones **1**–**10** has been investigated.

Ketones **2**, **5**, **7**, **8**, and **9** were synthesized by standard Grignard reactions, **3** and **6** by dialkylation of **1** and **4** with sodium hydride and methyl iodide, and **10** by the reaction of 3,3-dimethylacrylic acid with phenyllithium followed by conjugate addition of 2-propylmagnesium bromide in the presence of cuprous ion.[9] The ketones were purified by column chromatography or preparative vpc prior to photolysis. Quantum yields were determined on degassed 0.05 *M* benzene solutions irradiated to less than 5% conversion at 3130 Å using a merry-go-round apparatus and benzophenone–benzhydrol actinometers.[10] Quantum yields for type II elimination (Table I) are in reasonable

Table I. Quantum Yields and Kinetic Data for Photoelimination and Cyclization of Aryl Alkyl Ketones

	Ketone	Φ_{elim}	Φ_{cy}	Φ_{total}	% cy	$k_q\tau$, M^{-1}
1		0.36	0.042	0.40	10	670[a]
2		0.28	0.116 t	0.40	29	390
3		0.004	0.032	0.036	89	260
4		0.33	0.069 t 0.022 c	0.42	22	36[a]
5		0.17	0.094 t,t 0.030 t,c	0.29	43	37
6		0.012	0.044 t 0.018 c	0.074	84	44
7		0.26	0.045	0.30	15	245[a]
8		0.17	<0.005	0.17	<3	93
9		0.24	0.041	0.28	15	11[a]
10		0.014	~0.001	0.015	<10	5.6

[a] Values from ref 4a.

(1) The authors thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, The Research Corporation, and the Merck Foundation for support of this research.

(2) For a review, see P. J. Wagner and G. S. Hammond, *Advan. Photochem.*, 5, 21 (1968).

(3) P. J. Wagner, *J. Amer. Chem. Soc.*, 89, 5898 (1967).

(4) (a) P. J. Wagner and A. E. Kemppainen, *ibid.*, 90, 5896 (1968); (b) J. N. Pitts, D. R. Burley, J. C. Mani, and A. D. Broadbent, *ibid.*, 90, 5900 (1968); (c) P. J. Wagner and H. N. Schott, *ibid.*, 91, 5383 (1969).

(5) J. N. Barltrop and J. D. Coyle, *ibid.*, 90, 6584 (1968).

(6) F. D. Lewis and N. J. Turro, *ibid.*, 92, 311 (1970).

(7) F. D. Lewis, *ibid.*, 92, 5602 (1970).

(8) See ref 7 for the preceding paper in this series.

(9) J. A. Marshall and N. H. Andersen, *J. Org. Chem.*, 31, 667 (1966).

(10) W. M. Moore and M. Ketchum, *J. Amer. Chem. Soc.*, 84, 1368 (1962).