BIOORGANIC CHEMISTRY 9, 313–317 (1980)

Reactions of Vitamin B_{12r} with Polyhalogenated Hydrocarbon Pesticides

MAURO CESAR MARGHETTI LARANJEIRA, DANIEL W. ARMSTRONG, AND FARUK NOME

Departamento de Química, Universidade Federal de Santa Catarina, 88.000, Florianópolis, SC, Brasil

Received April 26, 1979

The reaction of vitamin B_{12r}, generated by photolysis of methylcobalamin under a nitrogen atmosphere, with 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), results in extensive dechlorination and formation of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) as the major products. Minor quantities of 1,1-bis(*p*-chlorophenyl)-2-chloroethane (DDMS), 1,1-bis(*p*-chlorophenyl)-2-chloroethylene (DDMU), 1,1-bis(*p*-chlorophenyl)ethane (DDO), and 1,1-bis(*p*-chlorophenyl)ethylene (DDNU) were also formed. Reaction of vitamin B_{12r} with DDD results in the production of DDMU and DDMS, the latter of which can react to produce DDNU and DDO. DDE and DDMU do not react with vitamin B_{12r}. The results obtained are suggestive of a vitamin B_{12r}-mediated dechlorination pathway for polyhalogenated hydrocarbon pesticides.

INTRODUCTION

Many kinetic and thermodynamic studies of a wide variety of reactions of vitamin B_{12} and related molecules in aqueous and micellar environments are available (*1–6*). Vitamin B_{12r}, which has been demonstrated to participate in many biochemical reactions (*2, 4, 7*) is a low-spin d^7 complex having one unpaired electron in the $3d_z2$ orbital (*1*). Thus, from a purely chemical point of view, it is an excellent candidate to react with alkyl halides via a free-radical dechlorination mechanism. Indeed it has been demonstrated that the reactions of pentacyanocobalt(II) with alkyl halides (*8, 9*), and those of cobaloximes(II) with benzylbromide in aprotic solvents (*10*) are likely to proceed via such a mechanism. Additionally, the products found in the metabolism of 1-1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT) in rat, based on analysis of liver and kidney tissue after massive DDT administration, are strongly suggestive of a free-radical dechlorination pathway (*11*). It has been demonstrated that in 0.1 *M* phosphate buffer pH 7.4 the reaction of DDT with vitamin B_{12r} resulted in the formation of 1,1-bis(*p*-chlorophenyl)-2,2-dichloroethane (DDD). This dechlorination was showed to proceed both in the presence and absence of light (*12*).

The experimental evidence described above, prompted us to study the reaction of vitamin B_{12r} with several polyhalogenated hydrocarbon pesticides such as DDT and derivatives, at longer reaction times and under stoichiometric conditions, in order to detect the possible formation of other products, and hence the viability of

0045-2068/80/030313-05$02.00/0

the participation of vitamin B_{12r} in the detoxication mechanism of these type of pesticides.

EXPERIMENTAL

All melting points are uncorrected. Infrared spectra were determined with a Perkin–Elmer 720 spectrophotometer. Nuclear magnetic resonance spectra were run in $CDCl_3$ on a Varian HA-100 spectrometer using Me_4Si as the internal standard. Ultraviolet spectra were obtained in methanol by means of a Varian 634 spectrophotometer.

Vitamin B_{12a}, aquocobalamin, was purchased from Merck. The compounds DDT, DDD, and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) were purchased from Aldrich Chemical Company (Golden Label), and their purity was found to be satisfactory by thin layer and gas chromatographic analysis. Synthesis of 1,1-bis(*p*-chlorophenyl)-2-chloroethane (DDMS) has already been described (*13*). The reagents 1,1-bis(*p*-chlorophenyl)-2-chloroethylene (DDMU) and 1,1-bis(*p*-chlorophenyl)ethylene (DDNU) were prepared by dehydrochlorination of DDD and DDMS, respectively (*13*); 1,1-bis(*p*-chloropheynl)ethane (DDO) was prepared by hydrogenation of DDMU at normal pressure using 10% PD/C as catalyst. Methylcobalamin was prepared reducing vitamin B_{12a} to vitamin B_{12s} with $NaBH_4$ followed by oxidative addition of CH_3I, as previously described (*6*). All the products were identified by comparison of their nmr and ir spectra with authentic samples.

All the reactions were carried out using equimolar amounts of methylcobalamin and the organic compound. In a typical reaction, 50 mg of DDT in 120 ml of methanol and 210 mg of methylcobalamin in 30 ml of water were mixed and the solution purged with purified nitrogen for 1 hr. The reaction mixture was then photolyzed with a 300-W tungsten lamp in order to generate vitamin B_{12r}. The reaction between vitamin B_{12r} and DDT was allowed to proceed for a period of 24 hr, and the organic fraction extracted with chloroform and rotary evaporated to dryness. A blank reaction carried out under identical experimental conditions, in the absence of vitamin B_{12r}, did not show any decomposition of DDT.

The organic fraction was analyzed by gas chromatography using a 2-m × 3.2-mm column packed with 3% OV-17 on 80- to 100-mesh Chrom-W-AW-DMCS, temperature of the column, detector, and injection block were 190, 200, and 215°C, respectively. Thin-layer chromatography was done using silica gel GF_{254} (Merck) as the adsorbent and petroleum ether, fraction 30–60, as developing solvent.

The reactions of DDD, DDMS, DDE, and DDMU with vitamin B_{12r} were carried out under identical experimental conditions. Identification of all the compounds was done by comparison of their respective retention times in gas chromatography and R_f values in thin-layer chromatography with authentic samples. In addition, the confirmation of the structure of the major products and the excess reactant, in each of the reactions, was performed by dehydrochlorina-

TABLE 1

REACTION PRODUCTS OF THE INTERACTION OF DDT AND VITAMIN B_{12r}

Component	Retention time (min)	R_f value	Percentage[a]
DDT	48.7	0.54	27.8(0.0)
DDD	39.5	0.36	29.5(0.0)
DDE	25.2	0.68	32.4(60.1)
DDMS	27.1	0.41	0.1(0.0)
DDMU	20.4	0.63	3.4(33.1)
DDO	11.4	0.62	0.1(6.7)
DDNU	15.0	0.87	6.7(6.7)

[a] Calculated from gas chromatographic data. The values in parentheses correspond to the same sample treated with alcoholic KOH.

tion with alcoholic KOH and ultraviolet and infrared spectroscopy of the compounds isolated by preparative layer chromatography.

RESULTS AND DISCUSSION

Vitamin B_{12r}, generated by the anaerobic photolysis of methylcobalamin, was found to react with the organic halides studied in stoichiometric proportion. Indeed, in all the reactions 1 eq of chloride ion was liberated in solution (determined by titration with silver nitrate in the presence of potassium chromate as indicator and by gravimetric analysis) for each equivalent of vitamin B_{12r} consumed. Vitamin B_{12a} was detected spectrophotometrically as the reaction product. The absorption maxima at 523, 497, and 350 nm which correspond to the α, β, and γ bands, respectively, were found to be identical with those already reported (*4*).

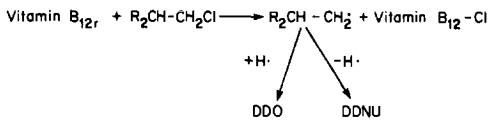
The results obtained in the analysis of the organic fraction in the reaction of DDT with vitamin B_{12r} are given in Table 1. DDD and DDE were found to be the major products.

Since the minor products (DDMS, DDMU, DDO, and DDNU) must be produced in consecutive reactions, we decided to study the behavior of all the reaction products with vitamin B_{12r} under similar conditions in order to clarify the possible reaction pathway.

The discrepancy between our findings and those previously described (*12*) can be easily explained, since we used a longer period of time in order to allow for the reaction go to completion. Our results are not contradictory with those reported by Berry and Stotter (*12*) but merely indicate that the reaction leads to the formation of not only DDD, but also several other products.

DDE and DDMU were found not to react with vitamin B_{12r}. This result is reasonable since vinyl halides are much less reactive than alkyl halides. Thus, DDE, DDMU, DDO, and DDNU (the two last compounds do not have aliphatic chlorine atoms) must be terminal products in any proposed reaction pathway.

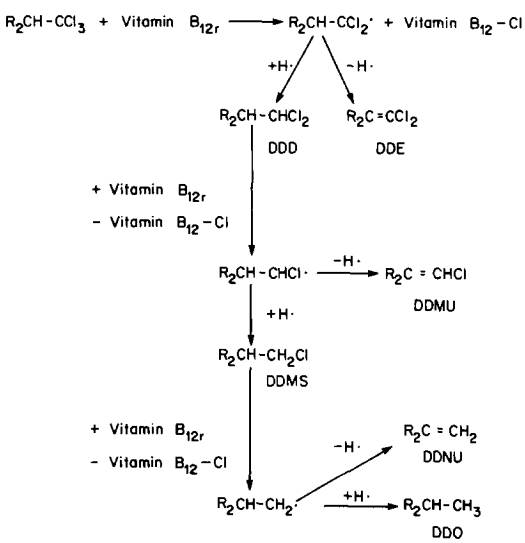
The reaction of DDMS with vitamin B_{12r} resulted in the production of DDO and DDNU and the liberation of chloride ion in solution. The appearance of the two products can be easily explained on the basis of a free radical type reaction (Eq. [1]).



[1]

It is important to remark that the vitamin B_{12}-Cl complex formed upon free radical abstraction of a chlorine atom is formally a Co(III) species. This electron transfer process explains the appearance of chloride ion in a stoichiometric amount, since the vitamin B_{12}-Cl complex readily becomes aquated forming vitamin B_{12a} and free chloride ion (*14*).

The interaction of DDD with vitamin B_{12r} is also consistent with the products found in the DDT reaction. Thus, DDMU, DDMS, DDNU, and DDO were the reaction products. Scheme I is consistent with all of our experimental evidence, and describes the reactions occurring in our system.



SCHEME I. R = *p*-chlorophenyl.

Vitamin B_{12r} reacts with DDT abstracting a chlorine atom and generating a free radical in the organic moiety. This organic free radical is able to lose or gain hydrogen yielding DDE and DDD, respectively. Reaction of vitamin B_{12r} with DDD will result in the production of DDMU and DDMS, the latter of which can further react to produce DDO and DDNU, thus explaining the formation of these compounds. *trans*-4,4′-Dichlorostilbene, a product which is formed in substantial amounts in the reactions of DDT with chromous chloride (*15*) and with Zn in hydrochloric acid (*16*), was not detected in the reaction of vitamin B_{12r} with DDT,

DDD, or DDMS. Interestingly, DDD reacts much slower with vitamin B_{12r} than DDT or DDMS. This result is consistent with the appearance of DDD as one of the major products in the reaction of DDT and also with the fact that *in vivo* DDD is detected as one of the major metabolites of DDT.

The results obtained are suggestive of a vitamin B_{12r}-mediated dechlorination pathway for polyhalogenated hydrocarbon pesticides and gives further credence to the involvement of metal centers in the dechlorination of DDT (*17*).

ACKNOWLEDGMENT

One of us (Faruk Nome) gratefully acknowledges CNPq for support of this work.

REFERENCES

1. W. C. RANDALL AND R. A. ALBERTY, *Biochemistry* **6**, 1520 (1967).
2. D. G. BROWN, *Progr. Inorg. Chem.* **18**, 177 (1973).
3. J. H. FENDLER, F. NOME, AND H. C. VAN WOERT, *J. Amer. Chem. Soc.* **96**, 6745 (1974).
4. F. NOME AND J. H. FENDLER, *J. Chem. Soc. Dalton Trans.* 1212 (1976).
5. F. NOME AND J. H. FENDLER, *J. Amer. Chem. Soc.* **99**, 1557 (1977).
6. G. C. ROBINSON, F. NOME, AND J. H. FENDLER, *J. Amer. Chem. Soc.* **99**, 4969 (1977).
7. H. RÜDIGER, *Eur. J. Biochem.* **21**, 264 (1971).
8. J. HALPERN AND J. P. MAHER, *J. Amer. Chem. Soc.* **87**, 5361 (1965).
9. J. KWIATEK AND J. K. SEYLER, *J. Organometallic Chem.* **3**, 421 (1965).
10. P. W. SCHENEIDER, P. F. PHELAN, AND J. HALPERN, *J. Amer. Chem. Soc.* **91**, 77 (1969).
11. S. DOONAN, "The Chemistry of the Carbon–Halogen Bond" (S. Patai, Ed.), Chap 13. Wiley, London/New York, 1973.
12. J. D. BERRY AND D. A. STOTTER, *Chemosphere* **6**, 783 (1977).
13. S. J. CRISTOL, N. L. HAUSE, A. J. QUANT, H. W. MILLER, K. R. EILAR, AND J. S. MEEK, *J. Amer. Chem. Soc.* **74**, 3333 (1951).
14. J. M. PRATT, "Inorganic Chemistry of Vitamin B_{12}." Academic Press, New York, 1972.
15. A. S. Y. CHAU AND W. P. COCHRANE, *Bull. Environ. Contam. Toxicol.* **5**, 133 (1970).
16. J. FORREST, O. STEPHENSON, AND W. A. WATERS, *J. Chem. Soc.*, 333 (1946).
17. D. A. STOTTER, *J. Inorg. Nucl. Chem.* **39**, 721 (1977).