

Inorganic Chemistry of Vitamin B₁₂

J. M. Pratt

B₁₂

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PREFACE

The rapid increase over the last decade in our knowledge of the co-ordination chemistry of the cobalt corrinoids, together with the stimulus this has given to the study of other cobalt complexes, has created the need for a source of reference on the subject. It is hoped that the present work will help to fill that need. For most sections of the book the literature has been covered up to the end of 1969; several additional papers published before September 1971, which provide substantial or significant new material, are mentioned in the Appendix. Some errors will doubtless have escaped detection and certain relevant items have been inadvertently omitted; I should be grateful if readers would let me know of any such errors or omissions.

I owe a debt of gratitude to a great many people; in particular to Dr. R. J. P. Williams, under whose supervision I carried out my D.Phil. research and began my interest in vitamin B₁₂; to Professor Dorothy Hodgkin and Drs. R. Bonnett, M. A. Foster, L. Mervyn and E. Lester Smith, who gave me the benefit of their advice on certain chapters; to Mr. S. C. Dyke for allowing me to make use of unpublished material on the history of pernicious anaemia; to two of my former research students, Drs. R. A. Firth and R. G. Thorp, who not only produced some of the experimental results recorded herein but who have also read and critized most of the book in the manuscript stage; to Mrs. Barbara Hunt for her excellent typing; and to the many other friends, colleagues and research students, too numerous to mention individually, who have helped with advice or contributed experimentally to our knowledge of the subject matter of the book. The writing of the book was begun while I was still at Oxford and completed after I joined Imperial Chemical Industries Limited. I should like to record my appreciation both to the Medical Research Council of London, who supported the work carried out at Oxford under the direction of Dr. R. J. P. Williams in the field of inorganic biochemistry, including vitamin B₁₂, and to the senior management of I.C.I.'s Petrochemical and Polymer Laboratory, who have given full support and encouragement to the writing of this book.

Runcorn, Cheshire.
September 1971

J. M. PRATT

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Section 1

Background

1

INTRODUCTION

I. The Chemistry of B_{12} in Historical Perspective	1
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Vitamin B_{12} or cyanocobalamin was isolated in 1948 in the form of dark red crystals. It is a diamagnetic, six-co-ordinate cobalt(III) complex. The molecule has the formidable empirical formula $C_{63}H_{88}O_{14}N_{14}PCo$; its structure is shown in Figs 1.1 and 1.2. The crystals contain a further 18–25 molecules of water of crystallization per molecule of B_{12} . (N.B. In this book we shall usually omit the word “vitamin” and refer to the compound simply as “ B_{12} ”).

1948 marks the beginning of the study of the chemistry of B_{12} and its derivatives (the cobalt corrinoids), to which contributions have been made by scientists in fields as far apart as microbiology and theoretical chemistry. The theme of this book is the inorganic chemistry, or more specifically the co-ordination chemistry, of the cobalt corrinoids. The aim of this chapter is to put the co-ordination chemistry of B_{12} into historical perspective and to provide references to reviews on other aspects of B_{12} chemistry (Section I). This leads on to a discussion of the aims and scope of the present book (Section II) and the way in which the material is arranged (Section III).

I. THE CHEMISTRY OF B_{12} IN HISTORICAL PERSPECTIVE

There can be few areas that have attracted the attention of a wider range of scientists than the study of B_{12} —from research workers in medicine and agriculture at one end of the spectrum through microbiologists, biochemists, organic chemists and co-ordination chemists to X-ray crystallographers at the other end; even theoretical chemists have been attracted by the problems of describing the electronic structure and interpreting the absorption spectra of B_{12} and its derivatives. It is interesting to observe the ebb and flow of activity in these different fields and to see how development in one area sparks off research in another.

The early history of B₁₂ belongs entirely to medicine. The disease, now known as pernicious anaemia, was first described in 1821. The next hundred years witnessed a slow but steady increase in our knowledge of the signs of the disease and methods of diagnosis but a total absence of any advance in the treatment of the disease, which remained incurable and was usually fatal. Then

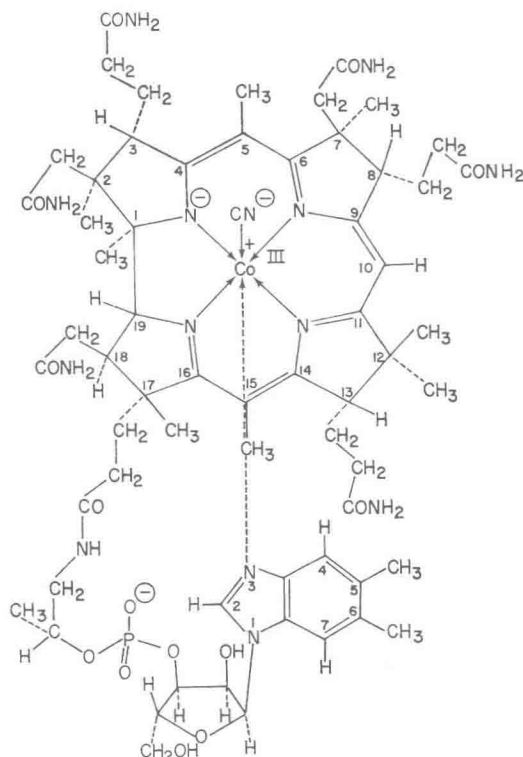


Fig. 1.1. Molecular structure of B₁₂. The positive charges of the cobalt(III) ion are balanced by the negative charges on the corrin ring, the cyanide and the phosphate.

in 1920, Whipple in California showed that the regeneration of red blood cells in dogs made anaemic by bleeding was stimulated by a diet containing liver, and in 1926 Minot and Murphy in Boston, Massachusetts reported a remarkable improvement in patients fed a diet of raw liver. This clue started the search for the "liver factor" or "anti-pernicious anaemia factor", which lasted over twenty years. Whipple, Minot and Murphy were awarded the Nobel Prize in Physiology and Medicine in 1934. The race to isolate the factor was very close. The isolation of crystalline vitamin B₁₂ was reported independently by two

teams in 1948, first by Folkers and his colleagues at Merck Laboratories in the U.S. (Rickes *et al.*, 1948) and then by Smith and Parker at Glaxo Laboratories in the U.K. (Smith, 1948; Smith and Parker, 1948), and in the following year by Ellis, Petrow and Snook at British Drug Houses in the U.K. (Ellis *et al.*, 1949).

1948 can be considered as the date which marks the beginning of the study of the chemistry of vitamin B₁₂. The role of B₁₂ and cobalt salts in medicine and agriculture is still being actively investigated, and some of these aspects are

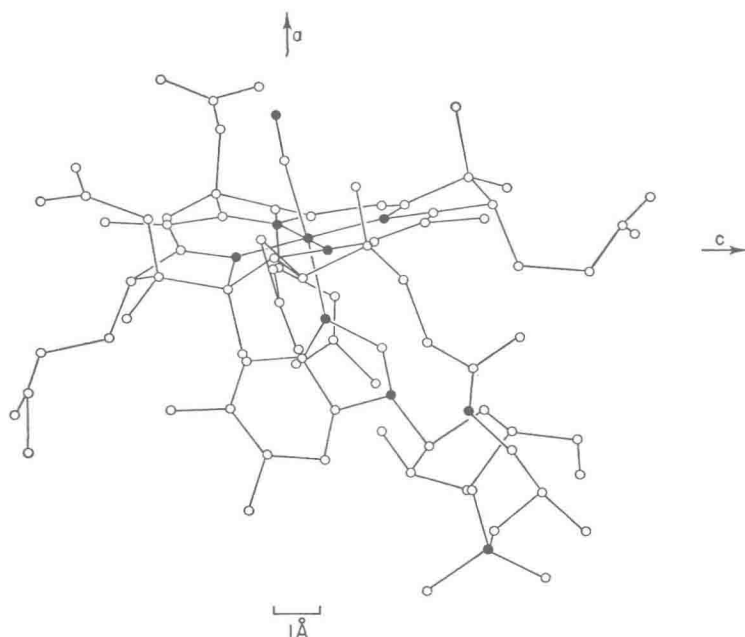


Fig. 1.2. Atomic positions in the molecule of B₁₂ viewed parallel with the crystallographic *b*-axis in crystals of wet B₁₂. The solid circles represent cobalt, nitrogen or phosphorus atoms. (From Brink-Shoemaker *et al.*, 1964.)

discussed in Chapter 3. From the chemists' point of view the most interesting developments in this area will be the elucidation of the exact role played by B₁₂ in metabolism in man and other animals.

Developments in the fields of biochemistry (including microbiology), organic chemistry, co-ordination chemistry and X-ray crystallography are much more closely linked and have probably been much more dramatic over the last twenty years than those in medicine and agriculture. One can, at the risk of being accused of oversimplification, attempt to pick out the main lines of research within these four related areas and their periods of maximum activity. After the original impetus received from medicine in 1926 the main stimuli have

come from the fields of microbiology and biochemistry, in which one can discern four successive and overlapping phases of activity. The first phase ended with the successful isolation of B₁₂ in 1948. The second phase (mainly 1948–1955, but still continuing) involved the development of methods for the large-scale production of B₁₂ by fermentation techniques through the screening and selection of micro-organisms; see the reviews by Perlman (1959) and by Mervyn and Smith (1964). It soon became clear that B₁₂ was only one of a family of closely-related compounds (corrinoids) occurring in nature, and the third phase (with peak activity from 1950 to 1962) may be considered as answering the question “which corrinoids occur in nature”; for fuller details of this phase see the reviews by Kon and Pawelkiewicz (1958), Perlman (1959), Mervyn and Smith (1964) and Wagner (1966). The most extensive work carried

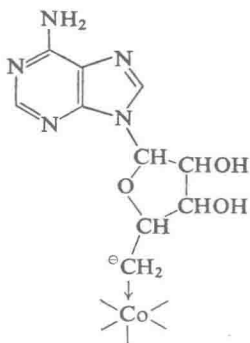


Fig. 1.3. The 5'-deoxyadenosyl ligand present in DBC and other coenzymes.

out under this heading was that of Bernhauer's group at Stuttgart (Bernhauer *et al.*, 1963, 1964), but the most important single discovery was the isolation by Barker and his associates in California in 1958 (Barker *et al.*, 1958) of the so-called “coenzyme” form of a corrinoid. When this was shown by X-ray analysis (Lenhert and Hodgkin, 1961) to possess the organoligand shown in Fig. 1.3, it led directly to a sharp increase in interest in both synthetic work for the preparation of organo-corrinoids and in the co-ordination chemistry of corrinoids in general. Barker's discovery of the coenzyme can also be considered as the start of the fourth phase of activity, that of enzymatic reactions, in which one aims to answer the questions “what reactions do corrinoids catalyse in conjunction with proteins and how”. The synthesis of methionine had been shown to require B₁₂ in one particular case in 1954, but the spate of work in discovering and studying the enzymatic reactions really got under way in 1958 and is still continuing. In no case has the mechanism of reaction yet been unravelled, but there can be little doubt from the quantity and quality of work being carried out in this field that the situation may soon be changed.

The enzymatic reactions are discussed in Chapter 17; see also the reviews by Weissbach and Dickerman (1965), Barker (1967), Stadtman (1967) and Hogenkamp (1968).

X-ray analysis, carried out by Professor Dorothy Hodgkin and her associates at Oxford, has played a very important part in the development of vitamin B₁₂ chemistry. Their work revealed the presence of two structures hitherto unknown in nature, namely the corrin ring which is present in all corrinoids (see Fig. 1.1) and the cobalt-carbon bond which is present in the coenzymes (see Fig. 1.3). The structure of the corrinoids which have been examined in detail also provide a unique insight into hydrogen-bonding, inter- and intramolecular interactions and subtle changes in configuration (e.g. of the corrin ring) in such complex molecules or co-ordination compounds. After the isolation of B₁₂ in 1948 the task of elucidating its structure was taken up by both organic chemists and by X-ray crystallographers. The two approaches were complementary. The classical degradative techniques used by the organic chemists provided evidence for the structure of the periphery of the molecule, while X-ray diffraction located the atoms near the cobalt atom in the centre. The structure of the corrin ring was announced by the X-ray crystallographers in 1954 (Brink *et al.*, 1954) and the complete structures of several corrinoids within the next few years. History repeated itself again shortly afterwards. The problem of determining the structure of the coenzyme, isolated by Barker in 1958, was taken up by both organic chemists and by X-ray crystallographers, but it was the latter who established the existence of the cobalt-carbon bond (Lenhert and Hodgkin, 1961). Although work on the X-ray analysis of the corrinoids began in 1948, the hey-day of crystallography (as far as the outside world was concerned) can probably be considered as lasting from 1954 into the late 1960's. Professor Hodgkin's elegant work in applying X-ray diffraction techniques to such complicated molecules was rewarded with the Nobel Prize for Chemistry in 1964. X-ray diffraction will probably be called in again in the future to elucidate the structure of the enzymatically active complexes formed between the corrinoids and proteins and hence provide information on finer points of detail in the mechanism of the enzymatic reactions. Professor Hodgkin has reviewed the work of her group (Hodgkin, 1958, 1964, 1965).

The first phase in the organic chemistry of B₁₂ was the determination of the structure, chiefly by classical degradative methods, which began immediately after the isolation of B₁₂ in 1948. Most of this work was carried out in the pharmaceutical firms by teams led by Folkers (Merck, U.S.), Smith (Glaxo, U.K.) and Petrow (B.D.H., U.K.) respectively and by Todd and his colleagues at Cambridge. This phase came to an end shortly after 1954, when the structure of B₁₂ was finally established by X-ray analysis (see above). For an account of this structural work see the reviews by Folkers and Wolf (1954), Smith (1955), Johnson and Todd (1957) and Bonnett (1963). The isolation of the coenzyme in

1958 again led to work on structural elucidation, which lasted until X-ray analysis in 1961 showed the coenzyme to be an organometallic complex with a cobalt-carbon bond. This in turn led to a great deal of work on the synthesis of organocorrinoids and on the properties and reactions of these compounds, which has continued unabated up to the present and which overlaps with work on the co-ordination chemistry of the corrinoids (see below). The last phase of organic chemistry, beginning in the early 1960's, has as its aim the total synthesis of the corrinoid structure with the correct substituents and stereochemistry found in the naturally occurring compounds. Johnson and his co-workers in the U.K. have been interested in the synthesis of corroles and tetrahydrocorrins, which differ from corrinoids in possessing extra double bonds in the periphery of the ring, while the problem of synthesizing the corrinoids themselves has been undertaken by Eschenmoser in Switzerland, Cornforth in the U.K. and Woodward in the U. S. Johnson's work is summarized in his review articles (Harris *et al.*, 1966; Johnson, 1967). Eschenmoser has summarized his earlier work in a review article and a later paper gives references to work up to and including 1969 (Eschenmoser *et al.*, 1965; Yamada *et al.*, 1969). Woodward gave an account of his work in 1968, but Cornforth's work has so far only been reported in lectures. See also the recent review by Melent'eva, Pekel' and Berezovskii (1969).

The last area of B₁₂ chemistry to gain momentum has undoubtedly been that of co-ordination chemistry. Co-ordination chemistry is here taken to mean essentially the properties and reactions of the cobalt atom and the axial ligands and includes the organo-metallic chemistry of the corrinoids, i.e. the chemistry of corrinoids in which one of the axial ligands is an organo-ligand. Although the co-ordination chemistry of the corrinoids has, in fact, been studied ever since the isolation of B₁₂ in 1948, the real upsurge in interest can be dated back to 1961 when Lenhert and Hodgkin (1961) showed by X-ray analysis that the coenzyme form of B₁₂ contained a cobalt-carbon bond and was therefore the first known naturally occurring organo-metallic compound. This discovery led to an immediate outburst of activity amongst organic chemists in synthesizing both the coenzyme itself and a wide range of other organo-corrinoids, which provided co-ordination chemists with a wealth of different unidentate organo-ligands (e.g. CH₃CH₂—, CH₂=CH—, HC≡C—, CH₃CO—, HOOC.CH₂—, etc.) whose properties and reactions could be investigated. It was soon shown that these organocorrinoids were diamagnetic and could be considered as cobalt(III) complexes containing a co-ordinated carbanion (e.g. CH₃[−] → Co^{III}). It also soon became clear that corrinoids which contained an organo-ligand or certain sulphur-bonded ligands (notably SO₃^{2−}) showed properties and reactions (e.g. spectra, formation constants for the binding of other ligands, photochemical reactions) which were very different from those of corrinoids containing the ligands more commonly studied by co-ordination

chemists such as H_2O , NH_3 , CN^- , NCS^- etc. As will be described in further detail in the final chapter the two main developments in the co-ordination chemistry of B_{12} have been in our knowledge and understanding of (A) organo-metallic compounds containing a σ -bonded carbanion ligand (in contrast to π -bonded ligands such as CO , olefins, acetylenes, cyclopentadienyl, etc.), and (B) the nature of the metal-ligand bond and *cis*- and *trans*-effects. The role of organo-corrinoids in enzymatic reactions has provided the stimulus for work under (A), while the existence of a wide range of easily handled corrinoids with different ligands and such different properties has stimulated research under (B). Results achieved with the corrinoids have, in turn, sparked off similar work on other cobalt(III) complexes. Contributions to the co-ordination chemistry of corrinoids have come from many laboratories throughout the world, and much of the work has been done by organic chemists, who have been interested primarily in the organometallic aspects. Of the work on co-ordination chemistry reported in the 1960's particular mention should be made of the contributions from the laboratories of Johnson in the U.K., of Bernhauer and his former colleagues in West Germany and of Hogenkamp in the U.S. The group at Oxford, including Williams and the author, represents the most inorganic wing of those involved in studying the co-ordination chemistry of the corrinoids.

It would probably be true to say that of the four areas of the chemistry of B_{12} delineated in this section (namely biochemistry, organic chemistry, X-ray crystallography and co-ordination chemistry) the greatest developments over the last decade have occurred within the areas of biochemistry and co-ordination chemistry. But, although the biochemistry has been well reviewed several times within the last few years, there has been no adequate review of the co-ordination chemistry. Some aspects of co-ordination chemistry have been included in the reviews by Bernhauer *et al.* (1963, 1964), Wagner (1966) and Hill *et al.* (1969) and information relating to the corrinoids has been included in a review on *cis*- and *trans*- effects in cobalt(III) complexes by Pratt and Thorp (1969). But there would appear to be a need for a more comprehensive review of the co-ordination chemistry of the corrinoids.

II. AIM AND SCOPE OF THE PRESENT WORK

The scope and treatment of the material in this book, as in any other, is determined mainly by

1. the need to have some unifying theme,
2. the readership it is intended to serve,
3. the existence of other reviews or books on similar topics and, of course,

4. personal preference and prejudice (for which any good writer should be able to find cogent reasons and arguments).

The theme of this book is the co-ordination chemistry of the cobalt corrinoids (vitamin B₁₂ and related compounds). It is useful to divide the corrinoid molecule (see Fig. 1.1) into the following four regions:

1. the axial ligands,
2. the cobalt atom,
3. the conjugated corrin chain of the equatorial ligand and
4. the side-chains; the outer ring of carbon atoms could be placed in either (3) or (4).

The co-ordination chemistry of the corrinoids concerns primarily regions (1) and (2). The importance of the corrin ring (region 3) resides mainly in the fact that the absorption spectra of the cobalt corrinoids are due to electronic transitions within the conjugated chain and provide a very sensitive indicator of changes at the cobalt atom due to changes in the valency or in the number and nature of the axial ligands. The few known "organic" reactions of the corrin ring are also of interest for the evidence they provide of *cis*-effects within the complex. As a broad generalization, therefore, one can say that the proportion of the total chemistry which is relevant to co-ordination chemistry falls and that to organic chemistry rises, as we progress from the centre (cobalt and axial ligands) out to the periphery (the side-chains). This will be reflected in the thoroughness with which the chemistry of the different regions is covered in this book.

The co-ordination chemistry of the corrinoids is probably of interest to several different groups of chemists, for example:

1. those already working in the field,
2. biochemists working on the enzymatic reactions of the corrinoids, who may obtain useful clues about the binding of the corrinoids to proteins and the mechanism of enzymatic reactions from studies on simpler systems,
3. biochemists interested in other metal complexes and metal-containing enzymes, in particular the iron porphyrins,
4. co-ordination chemists, working with other cobalt complexes, which often show similarities to the corrinoids,
5. co-ordination chemists in general, who may be interested in the contributions to the theory of the metal-ligand bond, which have come from the study of cobalt(III) corrinoids and
6. organometallic chemists, since the organocorrinoids have probably contributed more than any other group of complexes to filling the gap in our knowledge about the simplest possible type of transition metal organometallic complex, viz. the complex containing a unidentate σ -bonded carbanion.

For readers in groups 4–6 a short chapter has been included, which provides a brief sketch of the role of Vitamin B₁₂ in medicine and in nature in general (Chapter 3). The enzymatic reactions, which are catalysed by corrinoids in conjunction with a protein, are summarized in Chapter 17; in no case has the mechanism of reaction yet been completely elucidated. One might think there would also be a case for including a chapter on the theory of transition metal complexes, including crystal field theory: quite the reverse. The main contribution of B₁₂ chemistry to co-ordination chemistry has been to overthrow some of our most cherished existing theories and preconceived ideas, such as the importance of crystal field theory in correlating chemical properties, the kinetic inertness of cobalt(III) complexes and the instability of the bond between a transition metal and an alkyl group. The main contributions within the area of the co-ordination chemistry of B₁₂ which are of interest and relevance to co-ordination chemists and biochemists outside this area are summarized in the final chapter.

The way in which the chemistry of vitamin B₁₂ has been and still is developing, together with the available reviews, has been summarized in the preceding section. The only other book written on the subject is the valuable monograph called simply "Vitamin B₁₂" by E. Lester Smith, which was first published in 1957 and finally revised and brought out as the third edition in 1965; this concentrates mainly on the organic and medical aspects of vitamin B₁₂. It seems that the areas of biochemistry, organic chemistry and X-ray crystallography have been or are being adequately surveyed and reviewed, but that there is a real need to review the co-ordination chemistry of B₁₂. There is also undoubtedly scope for a new monograph on the subject of B₁₂, but to survey the whole field from medicine to X-ray crystallography would probably be too much for any single author and perhaps even too much to include in one book.

The aim of this book is, therefore,

1. to cover fairly thoroughly the co-ordination chemistry of B₁₂ (including the relevant areas of X-ray crystallography), i.e. to include all relevant material, to provide comprehensive references and a brief historical background and, where necessary, to discuss points at issue and to make suggestions and
2. to cover rather superficially the areas of organic chemistry and biochemistry, i.e. to provide only brief surveys together with references to reviews on the subject.

III. ARRANGEMENT OF THE MATERIAL

Both the collection and the organization of the information have presented problems. Information which is relevant to the co-ordination chemistry of the corrinoids is scattered through vast numbers of different journals, ranging