had m.p. 102–103°; λ_{max} 228 and 286 mμ (ε 12,100 and 4400); ν_{max} (Nujol) (cm.$^{-1}$) 3425, 1678 (α,β-unsaturated aldehyde and cyclopropyl ketone), 1658 (sh), and 1572; τ 0.3 (singlet, –CHO); 3.22 (singlet, olefinic H); 5.70 (broad peak, –CH–(OH)); 7.58 (singlet, OH); 7.80 (singlet, CH_3–(C=O)); 7.92 (singlet, olefinic $-CH_3$); 8.80, 8.98 (singlets, *gem*-dimethyl); 8.65, 9.2 (pair of multiplets symmetrical about their midpoint, A_2B_2 spectrum, 4 spirocyclopropane H).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12; O, 19.33; mol. wt., 248. Found: C, 72.62; H, 8.21; O, 19.75; mol. wt. (Rast), 265.

Under similar conditions the monoacetate of XI, m.p. 92–94° (λ_{max} 256 mμ), was allowed to react, though more slowly, with sodium metaperiodate, giving a cleavage product (λ_{max} 228 and 280 mμ); ν_{max} ($CHCl_3$) 1733 and 1698 cm.$^{-1}$.

Cleavage of XI with Manganese Dioxide. The triol XI (50 mg.), dissolved in chloroform (5 ml.), was stirred with activated manganese dioxide (0.5 g.)[13] for 3 hr. The mixture was filtered and the chloroform removed from the filtrate leaving a gum (λ_{max} 228 and 286 mμ) which crystallized completely on seeding with a crystal of XIV.

The monoacetate of XI, m.p. 92–94°, on similar treatment gave the same product as was obtained by cleavage with sodium metaperiodate. Illudin-M was unaffected by prolonged treatment (24 hr.) with activated manganese dioxide.

Acknowledgment. This work was supported by a grant (AI-00226) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

(13) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jensen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

The Hydrolysis of Vitamin B_{12}. Studies with Model Amides

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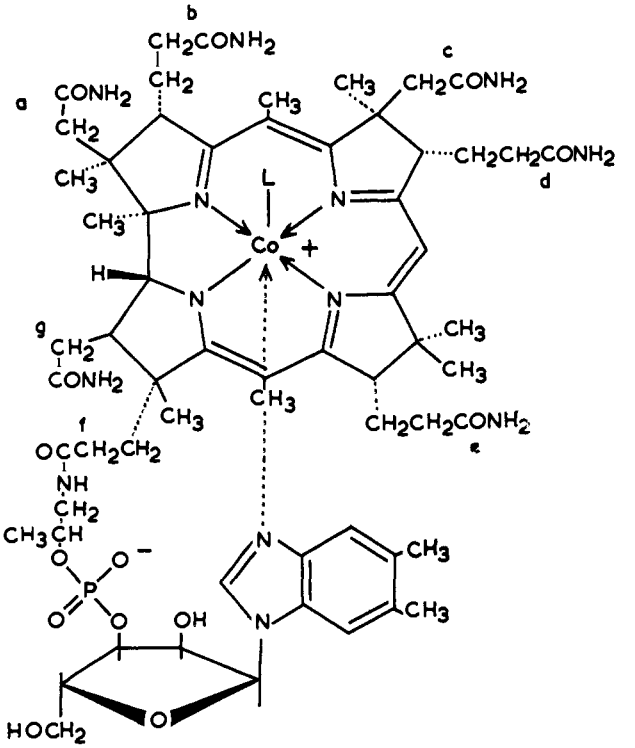
Contribution from the Department of Chemistry of the University of British Columbia, Vancouver 8, British Columbia, Canada, and Queen Mary College, London, E. 1, Great Britain. *Received October 28, 1964*

The suitability of certain cyclopentylacylamides as models for the steric situations of the amide groups in vitamin B_{12} is discussed. The syntheses of (1-methylcyclopentyl)acetamide, β-(1-methylcyclopentyl)propionamide, and β-(trans-2,2,3-trimethylcyclopentyl)propionamide are described, and the rates of hydrolysis of these and other model amides in aqueous hydrochloric acid–dioxane at 50° are recorded. Aquocobalamin has been hydrolyzed under identical conditions; comparison of these results with the model kinetics supports the earlier suggestion that steric factors have an important influence in the hydrolysis of vitamin B_{12}.

Introduction

The seven amide functions, two phosphate ester linkages, and ribosylamine group of vitamin B_{12} (I) lead to a complex fragmentation pattern when the vitamin is hydrolyzed.[1] The ribose–benziminazole bond, in fact, resists cleavage except under the most vigorous conditions, while methods which are fairly specific for the hydrolysis of the aminopropanol–phosphate linkage (leading to cobinamide) have been developed.[1,2] The present concern is the hydrolysis of the amide groups, a process which has been followed in detail[1] by the electrophoresis of the red acidic products. From this work it emerged that a considerable variation existed in the ease of hydrolysis of the amide groups; under mild acidic conditions (*e.g.*, 0.1 *N* HCl, room temperature, several days) three amide groups were cleaved to give mixtures containing three monobasic acids, three dibasic acids, and one tribasic acid, all of which retained the nucleotide. At the other extreme were two amide groups the hydrolysis of which required much more vigorous conditions (2 *N* HCl, 100°, 4 hr.).

A consideration of structure I suggests two major



I, L = CN, vitamin B_{12} (cyanocobalamin)
Ia, L = H_2O, aquocobalamin

(1) (a) J. B. Armitage, J. R. Cannon, A. W. Johnson, L. F. J. Parker, E. Lester Smith, W. H. Stafford, and A. R. Todd, *J. Chem. Soc.*, 3849 (1953); (b) for a review see R. Bonnett, *Chem. Rev.*, 63, 573 (1963).

(2) W. Friedrich and K. Bernhauer, *Z. Naturforsch.*, 9b, 685 (1954).

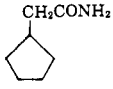
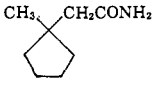
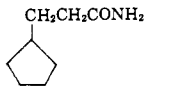
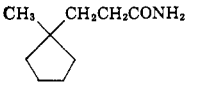
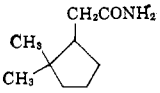
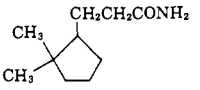
effects which could be responsible for variation in the rate of amide hydrolysis. An enhancement in rate might result from the participation of other structural features in the reaction. Among the groups which might participate are (i) the π-system of the corrin nucleus (*cf.* the anchimerically assisted solvolysis of 2-(Δ^3-cyclopentenyl)ethyl tosylate[3]); (ii) the cobalt atom, insofar as it can coordinate weakly with an amidic nitrogen and thus activate the adjacent carbonyl group (models suggest that neither of these processes is likely to be favored; they both require the acylamide groups to be bent back toward the center of the molecule, and although this may occur under certain circumstances, *e.g.*,[4] acetamide c in crystalline air-dried vitamin B_{12}, it is doubtful whether a significant interaction could arise without considerable steric strain. As far as coordination with the metal is concerned, this may be reasonably considered only for the longer chains (propionamides); the propionamides all lie on the same side of the macrocycle as the nucleotide, so that the observation[5] that the displacement of the latter has not occurred at an acid concentration (0.15 *N*) which cleaves the most labile amides argues against this mechanism.); (iii) other oxygenated functions; for example, the phosphate group might assist the hydrolysis of the propionamide groups, especially amides e and f.[6]

Additionally or alternatively the amide hydrolysis could be controlled by steric factors. Experiments with alkanoic acid amides[7] have shown that β-substitution causes a pronounced decrease in the rate of hydrolysis, while γ-substitution has much less effect. On this basis it has been suggested that the monocarboxylic acids obtained from the vitamin are propionic acid derivatives.[8] Further evidence for this conclusion is provided by the present study of selected model amides.

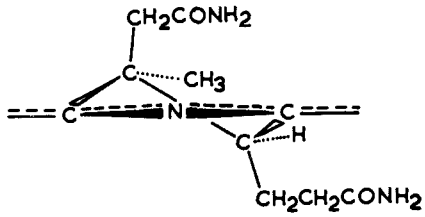
Selection of Model Amides. The extension of such generalizations as the "rule of six"[9] to the present case offers certain difficulties. These arise largely because the "rule" has been evolved primarily on the behavior of open-chain compounds, because few appropriate cyclic compounds have been studied quantitatively, and because it is difficult to estimate *a priori* what the steric effect of a ring position will be. Newman[9] has used the term "effective six number" (the number of atoms in the 6-position capable of yielding a coiled structure) in recognition of this difficulty. While the effect of ring size and strain on reaction at a ring atom has been extensively studied[10] relatively little is known, in quantitative terms, about substitution reactions in an alkyl chain attached to an alicyclic system.[11] Examples of reaction at a trigonal carbon atom (which are of chief interest here) are few. The effects of ring size on the rate of saponification of ethyl cycloalkylcarboxylates[12] and on the solvolysis of cycloalkylmethyl acetates[13] have been reported. In spite of its bearing on the chemistry of the monoterpenes and the naphthenic acids, the effect of ring substituents on the rate of solvolysis of side-chain carboxylic acid derivatives does not seem to have been evaluated. Since it is desired to examine this effect in relation to vitamin B_{12} hydrolysis, suitable model amides have been sought.

The model systems were selected to approximate the steric situations at the periphery of the macrocycle, but not to represent electronic effects of the unsaturated nucleus. The cyclopentane ring was taken as the basic system; while it has certain limitations (see below) it does fairly represent the tetrahedral arrangement at the β-positions, and with a suitable choice of substituents satisfactory models for individual β-positions can be obtained; see compounds II–V (Table I). In addition, models (such as VI and VII) which would allow the evaluation of the effect of geminal alkylation at the neighboring carbon atom were desirable. These simple models can scarcely be expected to represent the conformational detail found in vitamin B_{12}; thus,[4] ring **B** in the crystalline, air-dried vitamin has a conformation represented by VIII, whereas the model for, say, position 8 might be expected somewhat to favor conformation IX (C_s form). It is of interest that in the crystalline vitamin rings A, B, and C adopt the conformation in which two bulky groups are in the quasi-

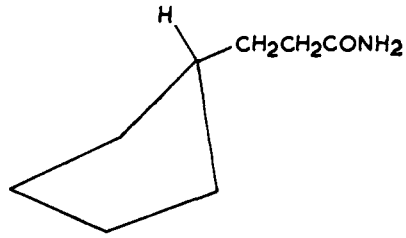
Table I. Model Amides

Compd.	Structural formula	Model for position
II		18
III		2, 7
IV		3, 8, 13
V		17
VI		18
VII		3, 8, 13

(3) P. D. Bartlett and S. Bank, *J. Am. Chem. Soc.*, **83**, 2591 (1961).
(4) D. C. Hodgkin, J. Lindsay, R. A. Sparks, K. N. Trueblood, and J. G. White, *Proc. Roy. Soc.* (London), **A266**, 494 (1962).
(5) P. George, D. H. Irvine, and S. C. Glauser, *Ann. N. Y. Acad. Sci.*, **88**, 393 (1960).
(6) K. Bernhauer, O. Müller, and F. Wagner in "Vitamin B_{12} und Intrinsic Faktor," H. C. Heinrich, Ed., Second Symposium, Ferdinand Enke Verlag, Stuttgart, 1962, p. 43; C. Zioudrou and G. L. Schmir, *J. Am. Chem. Soc.*, **85**, 3258 (1963).
(7) J. Cason and H. J. Wolfhagen, *J. Org. Chem.*, **14**, 155 (1949); J. Cason, C. Gastaldo, D. L. Glusker, J. Allinger, and L. B. Ash, *ibid.*, **18**, 1129 (1953).
(8) R. Bonnett, J. R. Cannon, V. M. Clark, A. W. Johnson, L. F. J. Parker, E. L. Smith, and A. Todd, *J. Chem. Soc.*, 1158 (1957).
(9) M. S. Newman, *J. Am. Chem. Soc.*, **72**, 4783 (1950).
(10) H. C. Brown, *J. Chem. Soc.*, 1248 (1956); C. H. Collins and G. S. Hammond, *J. Org. Chem.*, **25**, 698 (1960); T. C. Bruice and U. K. Pandit, *J. Am. Chem. Soc.*, **82**, 5858 (1960); O. H. Wheeler and M. A. Almeida, *J. Org. Chem.*, 27, 4448 (1962); H. C. Brown and F. J. Chloupek, *J. Am. Chem. Soc.*, **85**, 2322 (1963).
(11) E. E. Royals and A. H. Neal, *J. Org. Chem.*, **21**, 1448 (1956); C. F. Wilcox and S. S. Chibber, *ibid.*, 27, 2332 (1962); G. S. Skinner and F. P. F. Florentine, *J. Am. Chem. Soc.*, **76**, 3200 (1954).
(12) O. H. Wheeler, O. Chao, and J. R. Sanchez-Caldas, *J. Org. Chem.*, **26**, 2505 (1961).
(13) S. Sarel, L. Tsai, and M. S. Newman, *J. Am. Chem. Soc.*, **78**, 5420 (1956).



VIII



IX

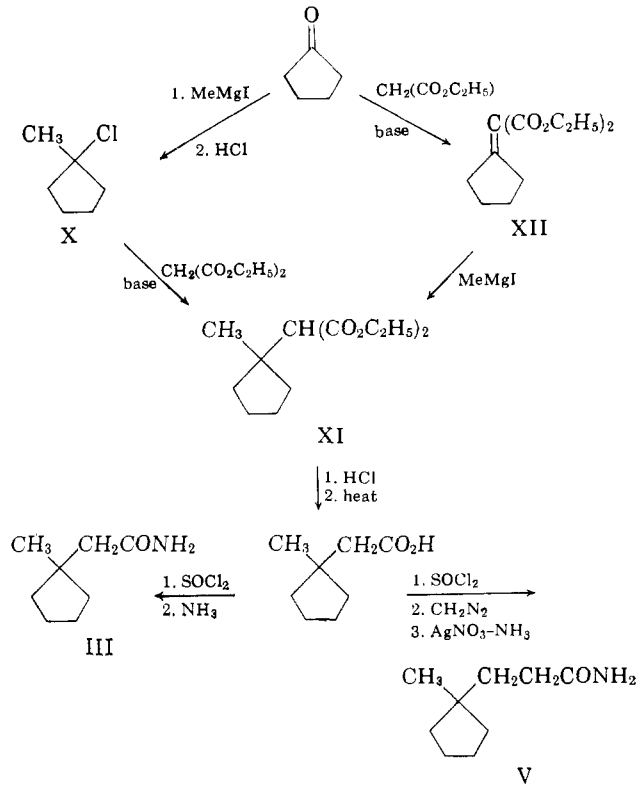
axial situation, an arrangement which presumably accommodates the steric effect of the methyl substituents at C-5 and C-15. However certain changes in the conformations of the β-positions have been observed in other corrinoids (*e.g.*, cobyric acid[14]), and even in the "wet" vitamin B_{12} crystal the situation is slightly changed.[15] It seems possible that in solutions of the vitamin the β-positions are somewhat flexible. Moreover, the energies involved in the interconversion of conformers of cyclopentanes[16] are expected to be smaller than those observed in the more familiar cyclohexane series, and since the projected hydrolyses are to be carried out at an elevated temperature, and the reacting group is in any case not directly attached to the ring system, it is considered that the conformational inequalities of the model compounds, both among themselves and with respect to the vitamin, are not likely to be significant compared with the gross steric effect of substitution.

Synthesis of Model Amides. Cyclopentylacetamide and cyclopentylpropionamide were readily available. The 1-methyl derivatives of these compounds were unknown and were synthesized from cyclopentanone, as shown in Chart I.

Two routes to diethyl (1-methylcyclopentyl)malonate (XI) were considered. The first involved attempted nucleophilic substitution of the tertiary halide X, where elimination would be expected to be favored. However, under suitable reaction conditions (extended reaction period at room temperature) the substitution proceeded to give the malonate XI in a yield which though low (13%) was acceptable in view of the availability of the starting materials. Understandably, there appear to be few examples of this type of preparation recorded; Dox and Bywater[17] report the reaction of *t*-butyl bromide and diethyl malonate to give the substitution product in 6.4% yield. The alternative route to XI involved the conjugate addition of methylmagnesium iodide to the unsaturated ester XII. With diethyl isopropylidenemalonate, this type of addition appears to proceed without difficulty,[18] but in the

(14) D. C. Hodgkin, private communication.
(15) C. Brink-Shoemaker, D. W. J. Cruickshank, D. C. Hodgkin, M. J. Kamper, and D. Pilling, *Proc. Roy. Soc.* (London), **A278**, 1 (1964).
(16) K. S. Pitzer and W. E. Donath, *J. Am. Chem. Soc.*, **81**, 3213 (1959).
(17) A. W. Dox and W. G. Bywater, *ibid.*, **58**, 731 (1936).
(18) G. A. R. Kon and E. A. Speight, *J. Chem. Soc.*, 2727 (1926); S. Wideqvist, *Chem. Abstr.*, **41**, 1615 (1947).

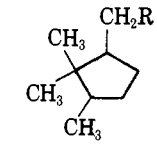
Chart I. Synthesis of (1-Methylcyclopentyl)acylamides



present case the product, presumably contaminated with 1,2-adducts, had to be purified by gas–liquid chromatography.

The remaining steps (Chart I) were straightforward. The n.m.r. spectrum of (1-methylcyclopentyl)acetamide (III) confirmed that rearrangement had not occurred during the reaction sequence; both the methyl signal (τ 8.91) and the deshielded methylene signal (τ 7.82) were sharp singlets.

In elaborating compounds of type VI and VII it was convenient to use camphor as the starting material, and thus employ α-campholanamide (XIII) as the model. It was considered that the 3-methyl substituent in XIII would be unlikely to interfere significantly with kinetic observations. *trans*-α-Campholanonitrile (XV) is reported to be available in one step by the



XIII, R = $CONH_2$
XIV, R = COOH
XV, R = CN
XVI, R = CH_2CONH_2

thermal cleavage of camphorimine in the presence of oxygen.[19] This interesting reaction is clearly quite complex; vapor phase chromatography shows that the more volatile fraction of the product consists of four components, *viz.* (i) camphor; (ii) a liquid, not observed by previous workers, the infrared spectrum of which closely resembles that of α-campholenonitrile; (iii) *trans*-α-campholanonitrile[19,20] (the major constituent); and (iv) a liquid, the infrared spectrum of which suggests a ketonitrile formulation (*cf.* the 3-

(19) F. Mahla and F. Tiemann, *Ber.*, **33**, 1929 (1900).
(20) P. Lipp, *ibid.*, **55**, 1883 (1922).

isopropyl-6-keto heptanonitrile reported by Mahla and Tiemann[19]). The camphor presumably arises by hydrolysis (either during the reaction—camphorimine is rather hygroscopic—or during work-up). The pathways by which the other components arise deserve further study, but, for the present purpose, this reaction, followed by preparative gas–liquid chromatography, proved to be a convenient route to α-campholanonitrile (XV) which on further elaboration gave both the acetamide XIII and the propionamide XVI derivatives required for the kinetic work.

Rate Measurements. The rates of hydrolysis of the six cyclopentylacylamides were observed under acidic (1.5 *N* HCl, aqueous dioxane, 50°) and basic (potassium hydroxide in propanol, at reflux temperature) conditions. In addition the hydrolyses of some open-chain analogs (XVII and XVIII) and of aquocobalamin (Ia) were examined under the acidic conditions. Aquocobalamin (Ia) rather than cyanocobalamin (I) was chosen for this work to avoid the interference of cyanide hydrolysis.

Experimental[21]

Cyclopentylacetamide, prepared from the corresponding acid in the usual way (thionyl chloride, ammonia), was recrystallized from benzene as white plates, m.p. 150–151° (lit.[22] m.p. 150°).

Anal. Calcd. for $C_7H_{13}NO$: C, 66.10; H, 10.30; N, 11.01. Found: C, 66.24; H, 10.30; N, 11.09.

β-Cyclopentylpropionamide similarly obtained had m.p. 124–125.5° (lit.[23] m.p. 122–123°).

Anal. Calcd. for $C_8H_{15}NO$: C, 68.04; H, 10.71; N, 9.92. Found: C, 68.06; H, 10.63; N, 9.90.

Diethyl (1-Methylcyclopentyl)malonate (XI). A. A mixture of 1-methylcyclopentyl chloride (152.5 g.) and diethyl malonate (206 g.) was added to an ethanolic solution of sodium ethoxide (33.6 g. of sodium in 600 ml. of anhydrous ethanol). The resulting solid mass was warmed and broken up, and then stirred for 5.5 days at room temperature. The volume of the liquid was reduced to ~250 ml. (rotary evaporator), water (400 ml.) was added, and the organic layer was separated. It was washed with salt solution, and, combined with an ethereal extract of the aqueous phases, was dried (Na_2SO_4) and distilled to give 40.8 g. (13%) of diethyl (1-methylcyclopentyl)malonate, b.p. 88.5–89.5° at 1.3 mm., $n^{20}D$ 1.4500.

Anal. Calcd. for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.67; H, 9.10.

Diethyl malonate (66%) was recovered.

B. To methylmagnesium iodide (from 8.6 g. of methyl iodide) cooled and stirred in ether (30 ml.) was gradually added diethyl cyclopentylidenemalonate[18] (14 g.) in ether (30 ml.). The mixture was refluxed for 10 min. and worked up[18] to yield 7.5 g. of a liquid, b.p. 98–106° at 2.3 mm. Redistillation gave 5.2 g., b.p. 98–100° at 2.2 mm., the infrared spectrum (film) of which showed an –OH band. Gas–liquid chromatography (Aerograph A-90-P, Apiezon J at 200°, helium carrier) separated three components of retention volumes 180 ml. (one part), 440 ml. (three parts), and 930 ml. (ten parts). The last component was collected. It had an infrared spectrum essentially identical with that of the product described under A above.

(1-Methylcyclopentyl)acetic Acid. Diethyl (1-methylcyclopentyl)malonate (9.17 g.) was refluxed (steam bath) with 10% ethanolic potassium hydroxide (40 ml.) for 13 hr. The cold solution deposited hygroscopic plates which were separated and dissolved in water (50 ml.). The acidified (dilute sulfuric acid) solution was continuously extracted with ether (3.5 hr.). The ether was removed and the residue was recrystallized from benzene to give 4.4 g. of (1-methylcyclopentyl)-malonic acid. A further 0.67 g. was obtained by making the alcoholic hydrolysate 30% in potassium hydroxide and refluxing for an additional 10 hr., yielding 5.07 g. (72%), m.p. 136–137°.

(1-Methylcyclopentyl)malonic acid (5 g.) was heated in an upright short-path distillation apparatus for 6 hr. at 160°; the evolution of carbon dioxide appeared to be complete in ~2.5 hr. The dark liquid was distilled to give 3.3 g. (86%) of (1-methylcyclopentyl)-acetic acid as a sour-smelling liquid, b.p. 74° at 0.22 mm.

Anal. Calcd. for $C_8H_{14}O_2$: C, 67.57; H, 9.93. Found: C, 67.29; H, 9.79.

(1-Methylcyclopentyl)acetamide (III). This was prepared from the acid chloride (b.p. 70–72° at 12 mm.) and formed white platelets, m.p. 96–97.5°, from benzene; n.m.r. spectrum (Varian A60, $CDCl_3$, internal standard tetramethylsilane): τ ~4.0 (broad, NH), 7.82 (singlet, $-CH_2CO-$), ~8.4 (multiplet, ring CH_2), and 8.91 (singlet, CH_3-).

Anal. Calcd. for $C_8H_{15}NO$: C, 68.04; H, 10.71; N, 9.92. Found: C, 68.14; H, 10.87; N, 10.18.

β-(1-Methylcyclopentyl)propionamide (V). (1-Methylcyclopentyl)acetyl chloride (5.8 g.) in ether (70 ml.) was added to an excess of a cold ethereal solution of diazomethane. The solution was allowed to warm up to room temperature. Evaporation of the ether gave ~5 g. of the crude diazo ketone which was dissolved in dioxane (150 ml.). Aqueous ammonia (*d* 0.88, 50 ml.) and 10% aqueous silver nitrate (10 ml.) were added. The mixture was heated for 1 hr. at 60–70° and, after the addition of more water (200 ml.), for an additional hour on the steam bath. The resulting suspension was treated with charcoal and filtered; the cold filtrate was saturated with sodium chloride and extracted with ether. Removal of the ether and crystallization (charcoal) from ethyl acetate gave 2.27 g. (42%) of β-(1-methylcyclopentyl)propionamide, m.p. 105–110°; recrystallized from benzene, m.p. 109–110°.

Anal. Calcd. for $C_9H_{17}NO$: C, 69.63; H, 11.04; N, 9.02. Found: C, 69.60; H, 10.97; N, 9.23.

trans-2,2,3-Trimethylcyclopentylacetonitrile (trans-α-Campholanonitrile) (XV).[19] A fine stream of air was bubbled for 10 hr. through molten camphorimine (85 g.) in an oil bath at 110–120°, the exit gases being passed *via* a condenser through a trap containing a small quantity of ethanol. The ethanol solution was concentrated and combined with the reddish brown reaction product, which was washed successively with dilute acid, dilute base, and water, and then steam distilled.

(21) Melting points are uncorrected. Analyses were by A. Bernhardt (Mülheim) and Mrs. Aldridge (Chemistry Dept., University of British Columbia).

(22) W. E. Doering and L. H. Knox, *J. Am. Chem. Soc.*, **78**, 4947 (1956).

(23) V. Zikan and M. Semonsky, *Collection Czech. Chem. Commun.*, **24**, 1274 (1959).

The distillate (~2.5 l.) was salted and extracted with ether, the extract being dried overnight ($CaSO_4$). Distillation gave two fractions, b.p. 104–118° at 20 mm. (21.7 g.) and b.p. 124–160° at 20 mm. (6 g.).

The experiment was repeated to obtain a total of 92 g. of the lower boiling fraction. This was further fractionated by preparative gas–liquid chromatography (Beckman Megachrome, Apiezon J at 215°, helium carrier). Four fractions were obtained: (i) camphor, m.p. 96°, oxime, m.p. 116°, m.m.p. 116–118°, 18% of the lower boiling fraction; (ii) a liquid, ν_{max} 2959 (s), 2237 (w), 1733 (w), and 800 (m) cm.$^{-1}$; 9% of the lower boiling fraction; (iii) *trans*-2,2,3-trimethylcyclopentylacetonitrile, b.p. 110.5° at 6 mm. (lit.[20] for inactive material b.p. 112–117° at 16 mm.), n^{20}D 1.4570 (lit.[19] nD 1.4611) 39.8 g. (69% of lower boiling fraction, 11% over-all); (iv) a liquid, ν_{max} 2985, 2242, 1715, and 1163 cm.$^{-1}$; 1% of lower boiling fraction.

trans-2,2,3-Trimethylcyclopentylacetamide (*trans-α-Campholanamide*) (*XIII*). *trans*-2,2,3-Trimethylcyclopentylacetonitrile (37.8 g.) was refluxed with 30% ethanolic potassium hydroxide for 24 hr. The ethanol was removed and the residue was dissolved in water; this solution was made strongly acidic (dilute sulfuric acid) and extracted with ether. The dried (Na_2SO_4) extract was distilled to give 35.5 g. (83%) of *trans*-2,3,3-trimethylcyclopentylacetic acid, b.p. 149–154° at 6 mm., n^{20}D 1.4628 (lit.[19] b.p. 160° at 22 mm., nD 1.4628). The corresponding amide was prepared in the usual way, yielding colorless plates, m.p. 145.5–146.5°, from benzene (lit.[19] 143° from ethyl acetate).

Anal. Calcd. for $C_{10}H_{19}NO$: C, 70.96; H, 11.32; N, 8.28. Found: C, 70.76; H, 11.26; N, 8.32.

(*trans-2,2,3-Trimethylcyclopentyl*)*propionamide* (*XVI*). This was prepared from *trans*-2,2,3-trimethylcyclopentylacetyl chloride, b.p. 98–100° at 15 mm., in an analogous fashion to the preparation of V above. This gave 24% (based on XIV) of the amide, white lustrous platelets, m.p. 83.5–84°, from benzene–petroleum ether (b.p. 60–80°).

Anal. Calcd. for $C_{11}H_{21}NO$: C, 72.08; H, 11.55; N, 7.64. Found: C, 72.09; H, 11.51; N, 7.89.

Other Amides. Isovaleramide (XVII) was obtained from the corresponding acid (thionyl chloride, ammonia) and recrystallized thrice from benzene–light petroleum ether (b.p. 60–80°). It was dried *in vacuo* at 50° for 4 hr., m.p. 136–137° (lit.[24] 136–137°).

Anal. Calcd. for $C_5H_{11}NO$: C, 59.37; H, 10.96; N, 13.85. Found: C, 59.62; H, 10.84; N, 13.81.

t-Butylacetamide (XVIII) was prepared and purified in a similar manner, m.p. 130–131° (lit.[25] 132°).

Anal. Calcd. for $C_6H_{13}NO$: C, 62.57; H, 11.38; N, 12.16. Found: C, 62.31; H, 11.31; N, 12.24.

Aquocobalamin, cyanocobalamin, and cobinamide were supplied by Glaxo Research Ltd. through the courtesy of Dr. E. Lester Smith.

Kinetic Measurements. The amide was dissolved in dioxane and the solution was then diluted to 4 volumes with 2 *N* hydrochloric acid ("1.5 *N* HCl in 25% aqueous dioxane"). An amide concentration of 0.05 *M* was used. The experiments were performed in duplicate in sealed tubes in a thermostatic bath at 50.0 ± 0.05°. At given intervals a tube was removed, cooled, and opened. Five milliliters of the solution was withdrawn and neutralized (*m*-cresol purple) with 1 *N* potassium hydroxide solution. The neutralized solution was buffered to pH 8.5 with borate buffer (0.05 *M*, 15 ml.) in a Markham still.[26,27] Steam distillation was continued for 10 min. and the liberated ammonia was trapped in 2% aqueous boric acid (20 ml.). The ammonia was determined by titration with 0.01 *N* HCl (methyl red–methylene blue mixed indicator). An initial blank was determined in all runs using amide solution which had not been heated to 50°. Titration values for total hydrolyses and for standard ammonium chloride samples were in accord with calculated values.

The hydrolyses of compounds XIII and XVI were accompanied by some separation of the corresponding acids; this did not appreciably alter the observed rate, however.

The hydrolysis of aquocobalamin was carried out in a similar way, except that an inert atmosphere was used. The aquocobalamin was not dried since it has been shown that this may cause slight decomposition.[28] Dioxane (25 ml.) was diluted to 100 ml. with 2 *N* hydrochloric acid and flushed with oxygen-free nitrogen for 0.5 hr. Aquocobalamin (150 mg., 18% hydration, 0.00365 *M*) was dissolved in 25 ml. of this solution and portions were sealed under nitrogen and treated as before. Ammonia was estimated with 0.002 *N* hydrochloric acid. Control experiments indicated that ammonia cobalichrome formation and the liberation of 1-aminopropan-2-ol did not interfere significantly with the determination. Titration values for standard ammonium chloride samples were in accord with the calculated values. The residue from each reaction tube was extracted by the phenol–ether method[1] and examined by paper electrophoresis (pH 6.5 and 10) for corrinoid carboxylic acids.[1] For comparison cobinamide and cyanocobalamin were hydrolyzed under the same conditions, and the progress of hydrolysis was followed by electrophoresis.

Results

Model Amides. All the simple amides gave good straight-line plots of t against log $a/(a - x)$ and the

Table II. Rates of Acid Hydrolysis of Amides in Aqueous Dioxane at 50° (1.5 *N* HCl)

Amide	First-order rate constant $k \times 10^4$ min.$^{-1}$ — First series	Second series	Average
II	13.4	13.3	13.3
IV	36.5	37.4	37.0
III	1.22	1.19	1.2
V	36.5	34.9	35.7
XIII	11.2	11.2	11.2
XVI	30.7	29.9	30.3
XVII[a]	6.65	6.77	6.7
XVIII[b]	0.94	0.96	0.95

[a] $(CH_3)_2CHCH_2CONH_2$. [b] $(CH_3)_3CCH_2CONH_2$.

(24) G. E. Philbrick, *J. Org. Chem.*, **19**, 623 (1954).
(25) A. H. Homeyer, F. C. Whitmore, and V. H. Wallingford, *J. Am. Chem. Soc.*, **55**, 4209 (1933).
(26) C. L. Wilson and D. W. Wilson, "Comprehensive Analytical Chemistry," Vol. 1B, Elsevier, Amsterdam, 1960, p. 500.
(27) J. M. Brierly, R. R. Sealock, and H. Diehl, *Iowa State Coll. J. Sci.*, **29**, 141 (1954).
(28) E. Lester Smith, *Analyst*, **87**, 183 (1962).

Table III. Acid Hydrolysis of Aquocobalamin in Aqueous Dioxane at 50° (1.5 *N* HCl)[a]

Time, hr.	NH_3[b] evolved, %	Unchanged B_{12a}	Cobinamide	Monoacid +Nt	Monoacid −Nt	Diacid +Nt	Diacid −Nt	Triacid +Nt	Triacid −Nt	Tetraacid	Pentaacid	Hexaacid	Heptaacid
0.5	10.1	XXX	X	XX		X							
1	14.6	XX	X	XX	X	XX							
2	23.7	XX	X	XX	X	XX	X	X					
3.5	34.4	X	X	XX	X	XX	XX	X	X				
6	44.2	X	..	X	X	XX	XX	XX	XX	X			
11	52.5	...	..	...	...	...	X	XX	XX	XX	X		
26	63.0	...	..	...	...	...	...	...	XX	XXX	XX	X	
53	65.0	...	..	...	...	...	...	...	X	XX	XXX	XX	
120	81.9	...	..	...	...	...	...	...	...	X	XXX	XXX	X
220	92.7	...	..	...	...	...	...	...	...	...	X	XXX	XXX

[a] XXX, major component; XX, moderate component; X, minor component. [b] Assuming 6 moles of NH_3.

Table IV. Acid Hydrolysis of Cyanocobalamin in Aqueous Dioxane at 50° (1.5 *N* HCl)[a,b]

Time, hr.	B_{12}	Cobinamide	Monoacid[c] +Nt	Monoacid[c] −Nt	Diacid[c] +Nt	Diacid[c] −Nt	Triacid, −Nt	Tetraacid
0.5	XXX	XX	XX	X	X			
1	XX	XX	XX	XX	X	X		
2	X	XX	XX	XX	X	XX	X	
3.5	...	X	X	XX	X	XX	XX	
6	...	...	...	XX	..	XXX	XX	X

[a] XXX, major component; XX, moderate component; X, minor component. [b] Polycarboxylic acids containing four or more carboxylic acid groups do not retain the nucleotide in any of the hydrolyses described in this paper. [c] +Nt, nucleotide containing; −Nt, nucleotide free.

hydrolyses were thus, as expected, first order with respect to amide. The rate constants are given in Table II; the deviations in these values, estimated from the worst plot in each graph, were not greater than 4%.

Corrinoid Derivatives. The results of the hydrolysis of aquocobalamin are indicated in Table III. The ammonia evolution was calculated on the basis of the six primary amide groups, it having been shown that 1-amino-2-propanol did not significantly interfere under the conditions used since it is much less steam volatile than is ammonia. The carboxylic acids were examined by paper electrophoresis[1] at pH 6.5 and 10. The amounts of individual fractions in relation to the total pigmented product were estimated visually and are recorded in Table III on a scale which is also used in Tables IV and V, which indicate the progress of hydrolyses of cyanocobalamin and cobinamide under the same conditions. In the last case the monocarboxylic acid fraction was isolated after hydrolysis (0.75 hr. in aqueous 1.5 *N* HCl at 50°) and shown by paper chromatography (butan-2-ol–aqueous ammonia (2:1) containing HCN, Whatman 3MM paper, downward development for 72 hr.) to consist of three components (ratio ~2:1:1) none of which was cobyric acid.

Table V. Acid Hydrolysis of Cobinamide in Aqueous Dioxane at 50° (1.5 *N* HCl)[a]

Time, hr.	Cobinamide	Monoacid	Diacid	Triacid	Tetraacid
0.5	XXX	XX	X		
1	XXX	XXX	XX		
2	XX	XXX	XX	X	
3.5	X	XX	XXX	XX	X
6	...	X	XXX	XXX	X

[a] XXX, major component; XX, moderate component; X, minor component.

Discussion

It is intended to discuss the trends observed in the hydrolysis of the simple amides, and then to relate these, where possible, to the hydrolysis of the vitamin B_{12} derivatives. It is assumed that the mechanism of hydrolysis is the same throughout the model series, *i.e.*, the rate-determining step will be the attack of water on the protonated amide function.[29] It may also be expected that for the models, differences in rate will be due essentially to steric factors. Polar effects in the acid hydrolysis of the related esters are known to be slight[30]; and, in any case, the effect would not be expected to be important here since the substituents occur at the β- or γ-positions, where, as the σ^* values indicate,[30] their polar influence is small.

Model Amides. The following conclusions emerge from the data in Table II.

(i) Cyclopentylacetamides are hydrolyzed more slowly than the corresponding cyclopentylpropionamides. In the examples which have no methyl group at the ring–chain junction (II and XIII compared with IV and XVI, respectively) the ratio of the rates is about 2.7.

(ii) Substitution of a methyl group at the β-position of the acetamide (II → III) results in a pronounced rate decrease by a factor of about eleven; in the corresponding propionamide case the lowering of the rate constant caused by γ-methylation (IV → V) is barely observable. These results have close analogies in the hydrolyses of

(29) M. L. Bender and R. D. Ginger, *J. Am. Chem. Soc.*, 77, 348 (1955).

(30) J. T. Edward and S. C. R. Meacock, *J. Chem. Soc.*, 2000 (1957); R. W. Taft, "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 556.

open-chain aliphatic amides[7]; certain modifications in detail do appear however. These are presumably associated with the compactness of the ring system and may now be mentioned.

(iii) γ- or δ-substitution has little effect on the hydrolyses of straight chain amides[7] or esters.[30] In the cyclopentylalkanoic acid system studied here, however, a *gem*-dimethyl group adjacent to the acylamide side chain (XIII, XVI) is associated with a small but definite decrease in rate. Presumably, in spite of the puckering possible in the cyclopentane ring, the acylamide function and one of the methyl groups can interact significantly. An effect of the same origin but of much greater magnitude is found in the esterification of the 2,5-dimethylcyclopentanecarboxylic acids.[31] It is of interest that the effect of adjacent substitution in the ring shows up even in the propionamide XVI.

(iv) The rate of hydrolysis of isovaleramide (XVII) is less than that of cyclopentylacetamide (II), while *t*-butylacetamide (XVIII) is hydrolyzed somewhat more slowly than is (1-methylcyclopentyl)acetamide (III). It appears that in this situation the steric effect of the cyclopentyl group is less than that of the isopropyl group; similar behavior has been observed in other systems (Chart II). This is somewhat surprising since the E_s values[30] (hydrolysis of RCOOR′) suggest that the cyclopentyl group (R; $E_s = -0.51$) exerts slightly more steric influence than the isopropyl group (R; $E_s = -0.47$). The differences involved are, however, quite small, and it is concluded that for the chain lengths under observation the steric effect of the cyclopentyl group is of the same order as that of the isopropyl group, but is smaller than that of the diethylcarbinyl group which is the strict open-chain analog of the five-membered ring. Formation of a five-membered ring places the ring members under a constraint, severely restricts the loci of the ring atoms and their immediate substituent atoms, and necessarily diminishes the total steric requirement.

(v) The alkaline hydrolysis of the six cyclopentylacylamides, carried out by the method of Cason and co-workers,[7] gave kinetic data (Table VI) which followed the same pattern as that already discussed. This confirms the importance of the steric effects revealed in the acid hydrolysis of the model amides.

Table VI. Rates of Basic Hydrolysis of Amides in Propanol at 95° (0.5 *N* KOH)

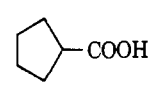
Amide	*k*, l. mole^{-1} hr.$^{-1}$	Amide	*k*, l. mole^{-1} hr.$^{-1}$
II	0.095	V	0.27
IV	0.30	XIII	0.090
III	0.011	XVI	0.27

Amide Hydrolysis in Cyano- and Aquocobalamins. In extensive studies on the hydrolysis of vitamin B_{12}, Todd and his co-workers[1] showed that the vitamin contained three readily hydrolyzed amide groups, while there was evidence for four more amide groups, two of which were rather resistant to hydrolysis. None of the three labile amides was the secondary amide f since the corresponding products retained the nucleotide. The nucleotide–propanolamine moiety could nevertheless be removed at a fairly early stage and was not associated with the two most resistant amide functions. As is apparent from Table III, the acid-catalyzed hydrolysis of aquocobalamin in aqueous dioxane follows the same general course.

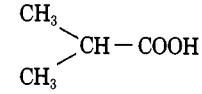
Clearly the model experiments strongly support the earlier suggestion[8] that the monocarboxylic acids are propionamide derivatives. The model propionamide IV (six-number[9] = 3), representing side chains b, d, and e, is hydrolyzed 30 times faster than the methylated acetamide III (total six-number = 9; effective six-number, *i.e.*, not counting ring carbons in six position, = 7) representing the amide groups at a and c, which are therefore considered to be the two groups most resistant to hydrolysis. The acetamide II without the β-methyl group is much less affected; it is hydrolyzed about 10 times faster than the highly hindered amide III and it is presumed, therefore, that amide g, to which it corresponds, is one of the two amides which are hydrolyzed at an intermediate rate. The other is then amide f. The model V shows that γ-methylation at the ring junction has only a slight hindering effect; a

Chart II. Relative Reaction Rates for the Analogous Cyclic and Open Chain Compounds[a]

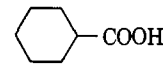
1. Esterification of carboxylic acids at 25° (α-branching)[b]



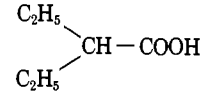
1.0



0.80

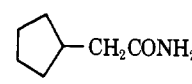


0.41

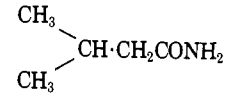


0.016

2. Acidic hydrolysis of amides at 50° (β-branching)

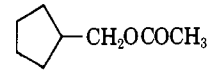


1.0

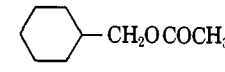


0.5

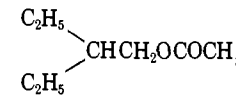
3. Alkaline hydrolysis of alkyl acetates at 20° (γ-branching)[c]



1.0



0.63



0.32

[a] In each series the rate of the specified reaction for the cyclopentyl derivatives is set at unity. [b] G. D. Advani and J. J. Sudborough, *J. Indian Inst. Sci.*, **6**, 41 (1923). [c] S. Sarel, L. Tsai, and M. S. Newman, *J. Am. Chem. Soc.*, **78**, 5420 (1956).

(31) T. L. Jacobs and W. H. Florsheim, *J. Am. Chem. Soc.*, 72, 261 (1950).

steric effect in the secondary amide function, which is not represented in the models, would also be expected (*cf.* the hindered alkyl acetates[32]). In addition there exists the possibility of an assisted cleavage of amide f; however, this appears to be important only under conditions of high acidity[33] and on the basis of the steric effects alone this amide would be expected to be hydrolyzed somewhat more slowly than the other three propionamides. The similarity of the hydrolysis product patterns for aquocobalamin (Table III) and cobinamide (Table V) suggests that the nucleotide does not have a profound effect on amide hydrolysis under these conditions.

Table VII. Relative Rates of Amide Hydrolysis

Model	Side chains represented	Relative rate of acid hydrolysis
II	g	11
IV	b, d, e	31
III	a, c	1.0
V	(f)	30

A semiquantitative relationship between the model amide hydrolyses and the hydrolysis of aquocobalamin must be somewhat arbitrary, but may be attempted. If the assumption is made that during the first 3.5 hr. only the three most labile amide groups of aquocobalamin are being hydrolyzed, then an apparent first-order rate constant for these groups ("k" = 55×10^{-4} min.$^{-1}$) may be extracted from the hydrolysis data (Table III). This value is to be compared with cyclopentylpropionamide $k = 37 \times 10^{-4}$ min.$^{-1}$; clearly the agreement is reasonable, and is improved by making allowance for the slow generation of ammonia from the other amides. After about 150 hr. essentially only the two most resistant amides remain (Table III). An apparent rate constant "k" = 1.1×10^{-4} min.$^{-1}$ may be obtained from this portion of the hydrolysis, and may be compared with the figure for (1-methylcyclopentyl)acetamide $k = 1.2 \times 10^{-4}$ min.$^{-1}$. Although it is not possible to compare individual rate constants, the similarities thus demonstrated between the extreme models and the extreme portions of the complex hydrolysis do suggest that the models chosen were suitable, and it follows that steric effects are of much importance in the hydrolysis of vitamin B_{12}.

Certain structural consequences may be mentioned briefly.[1b] It is apparent that the steric effects operate so as to reduce the number of isomeric acids which can be isolated; thus under normal hydrolytic conditions the mixture of monocarboxylic acids has three detectable components and not seven. Further support is now available for the view that the "antivitamins" (mono-N-substituted amides derived from the monocarboxylic acids) prepared by L. Smith[34] are propionic acid, and not acetic acid, derivatives. It is also of interest to consider probable structures for the pentacarboxylic acid(s) which can be isolated from vigorous alkaline hydrolysis of the vitamin.[35] This hydrolysis is complicated by the cyclization of acetamide c to give a fused lactam structure[1b] on ring B so that a hexacarboxylic acid is a product of total hydrolysis. The pentacarboxylic acid presumably has in addition the hindered acetamide residue at a; experiments are planned to elucidate whether or not this acetamide has also cyclized to give a lactam on ring A.

It is not to be concluded, of course, that in hydrolyses of such a complicated system steric effects alone will operate. Thus a comparison of Tables III and IV shows that cyanocobalamin, under the conditions employed, loses the nucleotide more rapidly than does aquocobalamin. This is presumably due to the greater *trans* effect of the cyano ligand[36] in labilizing the base–cobalt linkage; when this is broken chain f can adopt a conformation away from the corrinoid system and is thus more accessible to attacking reagents. Again, it has been found[1] that one of the three labile amide groups is more reactive than the other two. This is most readily observed under very mild conditions (*e.g.*, 0.02 *N* HCl, 20 days at ambient temperature) and might be due to an assisted hydrolysis. The acylamide chains are alternately up and down and may not interact significantly with one another, but participation of the nucleotide in the hydrolysis of amide e might be feasible.[6] Alternatively the distinction could be due to the steric effects of the methyl groups at C-5 and C-15 on the propionamides at the adjacent β-positions (this would result in propionamide d being the most labile). A third area where steric hindrance may not be dominant is the nucleotide–aminopropanol–propionic acid chain f, which could be involved in reactions assisted by neighboring groups. One instance is the cleavage of vitamin B_{12} under highly acidic conditions (which suppress amide hydrolysis[37]) to give cobinamide and the 2′- and 3′-ribonucleotides.[1,38] Other examples of neighboring group participation which result in fairly specific changes in this part of the molecule are at present being examined.

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(33) R. Bonnett and D. G. Redman, unpublished work.
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(37) T. W. J. Taylor, *J. Chem. Soc.*, 2741 (1930).
(38) R. Bonnett, J. G. Buchanan, A. W. Johnson, and A. Todd, *ibid.*, 1168 (1957).