ment of disappearance of unsaturation and of carbonyl content.[3] The reaction mixtures were prepared by adding 0.2 g. of aldehyde in 25 ml. of ethanol to 950 ml. of water and enough dilute hydrochloric acid to make the solution 1.8×10^{-3} *N* in hydrochloric acid. Unsaturation was followed by bromine titration, carbonyl content by reaction with hydroxylamine sulfate, titrating the liberated acid to *p*H 4.02 with 0.01 *N* barium hydroxide. Because of the high dilution of the dihydrocitral, the accuracy in the titrations was not better than about ±37. The results are summarized in Tables II and III.

TABLE II

RATE OF DISAPPEARANCE OF DIHYDROCITRAL AT 45° IN 1.8×10^{-3} *N* HYDROCHLORIC ACID

Time	Unsaturation,[a] %	Aldehyde,[b] %
0	100	100
24	69	89
48	55	86
62	50	...
72	...	90
84	48	...
240	43	64
2304	...	49

[a] By bromine titration. [b] By reaction with hydroxylamine sulfate.

TABLE III

RATE OF DISAPPEARANCE OF CITRAL AT 45° IN 1.8×10^{-3} *N* HYDROCHLORIC ACID

Time	Unsaturation,[a] %	Aldehyde,[b] %
8	49	53
48	12	24

[a] By bromine titration based on disappearance of only one of the two double bonds of citral. [b] By hydroxylamine sulfate.

The rates of disappearance of citral are in agreement with the rate predicted from the rate constant at 45° as measured by Price and Dickman.[3] As expected from the mechanism they proposed for citral and citronellal, the rate for dihydrocitral is much less, especially as measured by the disappearance of carbonyl. The difference in carbonyl and unsaturation titers for the dihydrocitral is unquestionably due to equilibrium hydration of the double bond conjugated to the aldehyde. If this be the explanation, the rate of hydration is considerably more rapid than for crotonaldehyde[4] and the equilibrium is slightly more favorable to the unsaturated aldehyde. Both these differences would be expected since in this case the product of hydration would be a tertiary, rather than a secondary, alcohol. The reason for the slow disappearance of the aldehyde group was not determined although the ease of oxidation and aldolization of aldehydes does not make it appear unreasonable.

Summary

A procedure has been described for the synthesis of several α,β-unsaturated aldehydes involving (a) addition of acyl chlorides to ketones to form β-chlorovinyl ketones, (b) reaction with methanolic alkali to form β-keto acetals, (c) dehydration of the hydroxy acetals over oxalic acid.

By this scheme, "dihydrocitral" was prepared and was shown to be far more stable in dilute aqueous acid than either citral or citronellal.

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[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

Studies of Some Characteristics of Vitamin B_{12b}

BY J. V. PIERCE, A. C. PAGE, JR., E. L. R. STOKSTAD AND T. H. JUKES

Introduction

The crystallization of vitamin B_{12b}, a biologically active substance related to vitamin B_{12}, was described previously.[1] The present communication describes further properties of the compound.

The crystalline vitamin was prepared from *Streptomyces aureofaciens* fermentation as before[1] and was dried at room temperature in a vacuum desiccator over phosphorus pentoxide. This procedure left about 6% of residual "moisture" which could be removed by heating at 100°; however, the data to be reported were obtained with unheated preparations.

The absorption spectra of vitamins B_{12} and B_{12b} in ultraviolet and visible light are compared in Fig. 1. Average analyses[2] of two samples showed the cobalt content to be 3.64% and the phosphorus content 2.0%, corresponding to a molecular weight of about 1500 on a moisture-free basis.[2a]

Infrared absorption spectra in mineral oil suspension were measured on both single beam and per cent. transmission instruments.[3] It was found that an absorption band at 2140 cm.$^{-1}$ exhibited by vitamin B_{12} was not shown by vitamin B_{12b}. An attempt to measure the refractive indices failed due to the opacity of the crystals.

The biological activity of vitamin B_{12b} for *Lactobacillus leichmannii* 313 and for chicks was found to be approximately the same as that of vitamin B_{12}.

Experimental

An aqueous solution of vitamin B_{12b}, 50 micrograms per ml., was examined in a Beckman spectrophotometer

(1) J. V. Pierce, A. C. Page, Jr., E. L. R. Stokstad and T. H. Jukes, THIS JOURNAL, **71**, 2952 (1949).

(2) Carried out by Mr. Paul Giesecke, Stamford Research Laboratories, American Cyanamid Company; see P. Giesecke, *Trans. Am. Inst. Mining and Metallurg. Engineers*, **169**, 706 (1946).

(2a) More recent observations with thoroughly dried samples of vitamin B_{12b} have resulted in values of 4.4% to 4.6% for cobalt and extinction coefficients ($E_{1\,cm.}^{1\%}$) of 136 at 274 mμ, 167 at 351 mμ and 57 at 525 mμ (J. A. Brockman, Jr., and J. V. Pierce, unpublished data).

(3) By Dr. R. C. Gore, Stamford Research Laboratories, American Cyanamid Company.

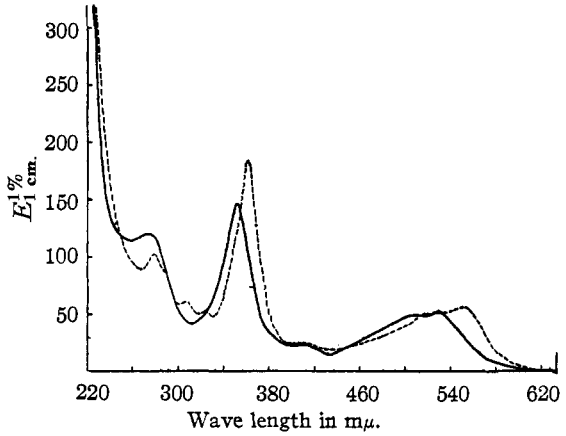

Fig. 1.—Absorption spectra of vitamins B_{12} (- - -) and B_{12b} (—) in ultraviolet and visible light.

at *p*H 5. The extinction coefficients ($E_{1\text{ cm.}}^{1\%}$) were 274 mμ, 119; at 351 mμ, 146; at 525 mμ, 52.[2a]

Assays were carried out with *L. leichmannii* 313 using the procedure described by Hoffmann and co-workers.[4] Vitamin B_{12} and vitamin B_{12b} produced growth responses, which were the same within the limits of variation of the assay method. In a typical experiment, B_{12} and B_{12b} at concentrations of 0.01 mγ per ml. of culture medium both produced growth responses corresponding to two-thirds of the maximum optical density obtained with B_{12} at higher levels.

Biological assay with chicks was carried out by the procedure of Stokstad and co-workers.[5] The data of an experiment are summarized in Table I. Twelve cross-bred Barred Rock × New Hampshire chicks were used in each group. The chicks were obtained from eggs laid by hens on a deficient diet as described previously.[5]

TABLE I

RESPONSE OF CHICKS ON 70% SOYBEAN MEAL BASAL DIET TO VITAMINS B_{12} AND B_{12b}

Group no.	Supplement injected weekly	Weight and no. of survivors (in parentheses) at 25 days
1	None	152 (5)
2	None	145 (4)
3	0.15 γ vitamin B_{12}	217 (11)
4	0.25 γ vitamin B_{12}	253 (11)
5	0.15 γ vitamin B_{12b}	205 (9)
6	0.25 γ vitamin B_{12b}	246 (12)

The results show that vitamin B_{12b} was approximately as effective as vitamin B_{12} in promoting growth and preventing mortality in chicks on the deficient diet.

Discussion

The name "vitamin B_{12a}" was given to a crystalline material which was prepared from vitamin B_{12} by catalytic hydrogenation.[6] The activity of vitamin B_{12a} was reported to be about 50% in the *L. lactis* assay, about one-half in the rat assay, and 30 ± 15% in the chick assay.[6] A clinical test with 25 micrograms of vitamin B_{12a} in a single pernicious anemia patient resulted in about 30% of a maximal hematological response. The activity of vitamin B_{12a} in the assay with *L. leichmannii* was expressed as 1 to 3 × 10^6 units/mg. for *L. leichmannii*. Presumably this "unit" refers to the liver standard described previously[7] and in terms of which vitamin B_{12} has an activity of 11 × 10^6 units/mg. This indicates that the activity of vitamin B_{12a} is between 9 and 27% of that of vitamin B_{12} for *L. leichmannii*, agreeing with a value of 20% mentioned elsewhere.[8] In the present investigation the activity of vitamin B_{12b} appeared to be approximately as great as that of vitamin B_{12} in assays with *L. leichmannii* and with chicks while preliminary studies with rats[9] indicated that the activity of vitamin B_{12b} was as great as that of vitamin B_{12}. Studies by Lichtman and co-workers[10] have shown that vitamin B_{12b} is effective parenterally in the treatment of patients with Addisonian pernicious anemia in amounts of 1 to 2 micrograms daily.

It was stated by Laland and Klem[11] that the ultraviolet absorption spectrum of their concentrated preparation of anti-pernicious anemia factor showed inflections at 250–265 mμ and at 345–350 mμ. This early observation is of much interest in view of the absorption spectra of vitamins B_{12} and B_{12b}.

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Summary

Vitamin B_{12b} was crystallized from *Streptomyces aureofaciens* fermentation and its absorption spectrum was compared with that of vitamin B_{12}. The cobalt and phosphorus content of vitamin B_{12b} were about 3.64 and 2.0%, respectively. About 6% of residual moisture was present after drying *in vacuo* at room temperature.

A difference between vitamins B_{12} and B_{12b} was noted in the infrared absorption spectra at 2140 cm.$^{-1}$.

The biological activity of vitamin B_{12b} was found to be approximately as great as that of vitamin B_{12} in assays with *L. leichmannii* and with chicks.

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