MODELING THE BIOCHEMICAL FIXATION OF CO_2 BY BIOTIN COENZYME

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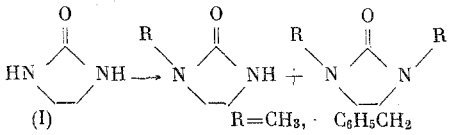
One of the functions of the biotin coenzyme (BC) is the fixation of CO_2, which leads to the formation of N-carboxy-BC [1]. Attempts have been made in recent years to reproduce this biochemical reaction under chemical conditions employing 2-imidazolidinone (I) as the BC model. In the free state (I) lacks noticeable nucleophilicity [2], whereas its O-methyl derivative reacts with certain electrophilic compounds, for example, the 4-nitro- and 2,4-dinitrophenyl acetates [3]. Literature data are absent on the possibility of the nonenzymatic N-carboxylation of either (I) or biotin (II).

In the present paper we studied the N-carboxylation of (I), (II), and desthiobiotin (III) under the influence of CO_2 and magnesium methyl carbonate (IV) [4-6]. In order to find the optimum reaction conditions we first studied the ability of the (I) salts to react with CH_3I and $C_6H_5CH_2Br$ in CH_3OH, t-C_4H_9OH, dioxane, DMF, and toluene. Here the most complete alkylation of the ambident (I) salts occurs when either t-C_4H_9OK in t-C_4H_9OH or NaH in dioxane are used as the metalating agents. However, even when using one equivalent of the metalating agent, either in t-C_4H_9OH or in dioxane, the ambident (I) salts give a mixture of the N-alkyl- and N, N'-dialkyl-(I).



The reaction of the K salt of (I) with CS_2 in t-C_4H_9OH also proceeds in a nonselective manner, and leads to a mixture of addition products (V) and (VI), which were characterized as the corresponding S-benzyl derivatives (VII) and (VIII).

The alkylation and dithiocarboxylation of (I) cannot be effected when CH_3ONa in CH_3OH is used, while decomposition of the solvent occurs when (I) is treated with NaH in DMF, with the liberation of $(CH_3)_2NH$. In order to effect the N-carboxylation of (I) with CO_2 we used the system t-C_4H_9OK–t-C_4H_9OH. It proved that the K salt of (I) in t-C_4H_9OH adds CO_2 at a high speed at ~20° and a slight excess pressure.

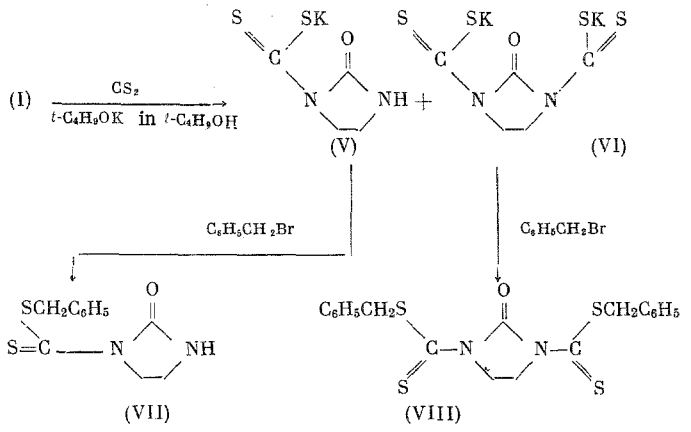


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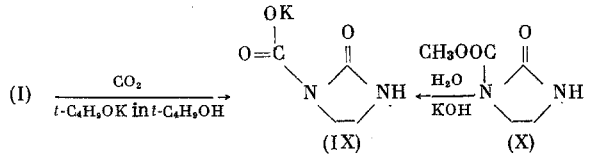
TABLE 1

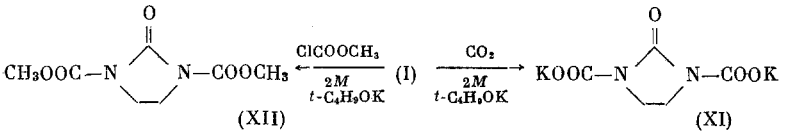
IR spectrum*	Compound														
	I	II	III	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	Mg salt (II)	Mg salt (III)	XVII
ν_{CO}, cm^{-1}	1660	1652 1695 1712	1660 1710	1742	1742	1635 1685 1740	1675 1760	1715 1780	1725 1780	1665 1712	1662 1675 1680 1692 1712	1712	1660 1692	1675 1696	1664

*Taken on a UR-20 instrument in KBr.

The structure of the K salt of N-carboxy-(I) (IX) that is formed here was confirmed by its decarboxylation under the influence of dilute HCl solution and conversion to the starting (I), and also by a coinciding of the IR spectra of (IX) (Table 1) and an authentic specimen, which was obtained by the saponification of N-carbomethoxy-(I) (X) [2].

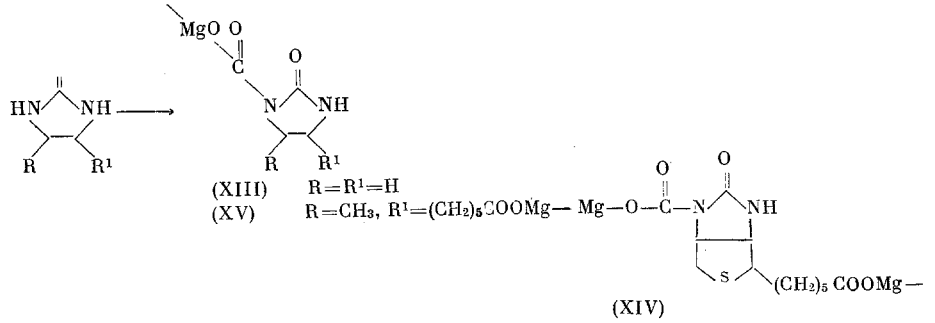


The di-K salt of N, N'-dicarboxy-(I) (XI) can also be obtained from (I), provided the reaction with CO_2 is run in the presence of two moles of $t\text{-}C_4H_9OK$.



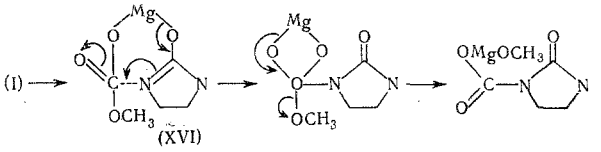
The reaction of (I) and $ClCOOCH_3$ under analogous conditions gives N, N'-dicarbomethoxy-(I) (XII). As can be seen from the data in Table 1, the insertion of additional electron-acceptor groups into the (IX) and (X) molecules causes a substantial shift of the carbonyl bands toward higher frequencies. In view of the low solubility of the K salts of (II) and (III) in $t\text{-}C_4H_9OH$ and dioxane the N-carboxylation of (II) and (III) could not be run in these solvents.

The ambident (I) salt practically loses its ability to react with CO_2 when the system $t\text{-}C_4H_9OK - t\text{-}C_4H_9OH$ is replaced by the system $CH_3ONa - CH_3OH$. However, judging by the appearance of a weak band at 1740 cm^{-1}, which is characteristic for the $O=\overset{O-}{\underset{|}{C}}-\underset{|}{N}-\overset{O}{\overset{||}{C}}-\underset{|}{N}-$ group, the addition of CO_2 to (II) proceeds to a slight degree in the presence of CH_3ONa in CH_3OH. The most suitable reagent for the N-carboxylation of the cyclic derivatives of urea proved to be (IV), which at 50-100°, in DMF medium, reacts with them in 5-6 h. The structure of the Mg salts (XIII)-(XV) that are formed here was confirmed by hydrolysis and decarboxylation to the starting (I)-(III), and also by the IR spectra, which contain the band of the $O=\overset{O-Mg-}{\underset{|}{C}}-\underset{|}{N}-\overset{O}{\overset{||}{C}}$ group at 1712 cm^{-1}. (II) and (III) do not react with CO_2 in the presence of $(CH_3O)_2Mg$ in CH_3OH, while (I) gives only traces of (XIII)



The position of the $-Mg-O-\overset{O}{\overset{\|}{C}}-$ group in the molecules of the unsymmetrical derivatives (I)-(XIV) and (XV) was adopted with the steric hindrance, introduced by the polymethylene chainlet, taken into account [7, 8].

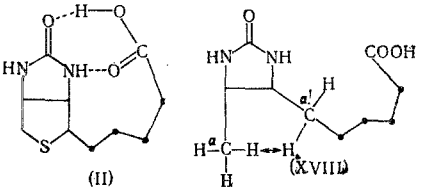
The beneficial effect of Mg^{2+} on the carboxylation of (I)-(III) can be explained by the formation of a cyclic transition state of the (XVI) type, which facilitates nucleophilic attack of the nitrogen atom by the carbon atom of the CO group



(XVI)

It is interesting to mention that the involvement of Mg^{2+} ions is also necessary for the enzymatic carboxylation of (II) [9]. Important differences in the ability of (I)-(III) to undergo nonenzymatic carboxylation cannot be detected, although based on the data of the NMR spectra, (II) should have a higher NH acidity than (I) and (III). Here the acidity of the urea derivatives decreases in the order: (II) > (I), (III) > N, N'-dimethylurea (XVII), since the δ_{NH}* for these compounds is respectively equal to 6.30 and 6.37, 6.14, 6.12, and 5.66 ppm.

The higher NH acidity of (I)-(III) when compared to (XVII) must probably be regarded as being due to the favorable effect of the cyclic structure on the proton lability of the endocyclic hydrogen atoms. The fact that (II) has a higher NH acidity than (I) and (III) can be attributed to the closeness of the $-NH-CO-NH-$ and $-COOH$ groups and the formation of intramolecular hydrogen bonds between them [7]. Such an arrangement of the tetramethylene chainlet in (II) is in agreement with the data of the x-ray structure analysis [10, 11], and also of the IR and NMR spectra, which contain the split bands of the CO groups and the split signals of the H_N protons. A similar splitting of the bands of the CO groups and of the signals of the H_N protons is not observed in the spectra of (III), for which the "contiguous" conformation of (XVIII) should be energetically more favorable due to the repulsion of the α- and α'-hydrogen atoms



(II) (XVIII)

Not excluded is the fact that the high NH acidity of (II) plays a beneficial role in the process for the formation of N-carboxy-BC under enzymatic conditions.

EXPERIMENTAL

Methylation of 2-Imidazolidinone (I). a) With stirring, to a solution of t-C_4H_9OK (from 0.24 g of K and 10 ml of t-C_4H_9OH) was added 0.5 g of (I), the mixture was allowed to stand at ~20° for 12 h, 1.2 ml of CH_3I was added, the mixture was refluxed for 8 h, filtered, the filtrate was evaporated in vacuo, and the residue was chromatographed on a plate covered with Al_2O_3 (here and subsequently III activity) in the system: acetone–hexane, 1 : 1. Here we obtained 0.24 g (41%) of N-methyl-(I), mp 99-102°, R_f 0.58 [12] (here and subsequently TLC on Al_2O_3, acetone–water, 9 : 1, detection with iodine vapors), and 0.29 g (44%) of N, N'-dimethyl-(I); n_D^{21} 1.4660, R_f 0.78 [8].

b) To a solution of 0.5 g of (I) in 20 ml of dioxane was added 0.28 g of a 50% suspension of NaH in Nujol. The mixture was heated at 70-80° for 6 h, 1.5 ml of CH_3I was added, the mixture was refluxed for 8 h, cooled, filtered, and the filtrate was evaporated. After the above indicated workup we obtained 0.10 g (17%) of N-methyl-(I) and 0.32 g (48%) of N, N'-dimethyl-(I).

Benzylation of (I). a) To a solution of the K salt of (I) (from 0.24 g of K and 0.5 g of (I) in 10 ml of t-C_4H_9OH) was added 0.7 ml of $C_6H_5CH_2Br$ and the mixture was refluxed for 10 h. The filtrate was evaporated in vacuo and the residue was diluted with 3 ml of ether. We obtained 0.16 g (15.4%) of N-benzyl-(I), mp 125-126°; R_f 0.66 (acetone–water, 20 : 0.2); cf. [13]. From the ether mother liquor, after preparative

*The NMR spectra were taken in DMSO solution on an RS-60 instrument using HMDS as the standard (the signals are broad).

TLC on Al_2O_3 in the system: hexane–acetone, 7 : 2, was isolated an additional 0.07 g (7%) of N-benzyl-(I) and 0.33 g (21%) of N, N'-dibenzyl-(I), R_f 0.47 (hexane–acetone, 3 : 1), mp 91-93°; cf. [14].

b) A mixture of the Na salt of (I) [from 0.28 g of a 50% suspension of NaH in Nujol and 0.5 g of (I)] and 0.7 ml of $C_6H_5CH_2Br$ in 20 ml of dioxane was refluxed for 10 h, and then worked up as described above. Dilution of the residue with 8 ml of ether gave 0.21 g (20%) of N-benzyl-(I) with mp 123-124°. From the mother liquor by preparative TLC on Al_2O_3 was isolated 0.21 g of N, N'-dibenzyl-(I), mp 90-91°.

Reaction of K Salt of (I) with CS_2. a) To a solution of the K salt of (I) (from 0.37 g of K and 1 g of (I) in 20 ml of t-C_4H_9OH), with stirring, was added 1 ml of CS_2, and after 6 h was added 1.4 ml of $C_6H_5CH_2Br$. The mixture was allowed to stand for 12 h, evaporated in vacuo, the residue was diluted with water, and the obtained precipitate was successively recrystallized from a mixture of 60 ml of benzene and 10 ml of n-hexane, and from a mixture of 15 ml of alcohol, 20 ml of benzene, and 5 ml of chloroform. We obtained 0.28 g (6%) of the bis-dithio derivative (VIII), mp 218-219°, R_f 0.90 (acetone–hexane, 1 : 1). Infrared spectrum (KBr): 1742 cm^{-1}. Found: C 54.49; H 4.39; N 7.10; S 30.43%. $C_{19}H_{18}ON_2S_4$. Calculated: C 54.54; H 4.31; N 6.69; S 30.62%. From the mother liquors, after preparative TLC on Al_2O_3 in the system: acetone–hexane, 1 : 1, was isolated 0.32 g (12%) of the monothio derivative (VII), mp 177-178°, R_f 0.38 (acetone–hexane, 1 : 1). Infrared spectrum (KBr): 1742 cm^{-1}. Found: C 51.87; H 4.82; S 25.68%. $C_{11}H_{12} \cdot ON_2S_2$. Calculated: C 52.34; H 4.76; S 25.39%.

b) To a solution of the K salt of (I) (from 0.37 g of K and 0.5 g of (I) in 10 ml of t-C_4H_9OH) was added 1 ml of CS_2, and after 5 h was added 1.4 ml of $C_6H_5CH_2Br$, the mixture was allowed to stand 12 h, evaporated, the residue was diluted with water, and the obtained product was filtered. We obtained 0.9 g (38%) of (VIII), mp 215-216° (from benzene).

Carboxylation of (I). a) A solution of the K salt of (I) (from 0.24 g of K and 0.5 g of (I) in 7 ml of t-C_4H_9OH) was stirred in a CO_2 atmosphere (1.1 atm) at ~20° for 30 min, and then it was diluted with absolute ether. We obtained 0.88 g of (IX), the K salt of N-carboxy-(I). The treatment of 0.88 g of (IX) with excess dilute HCl solution gave 0.32 g of (I).

b) The treatment of (I) with CO_2 in the presence of 2 M of t-C_4H_9OK in t-C_4H_9OH, as described above, gave (XI), the di-K salt of N, N'-dicarboxy-(I).

c) A mixture of magnesium methyl carbonate (IV) [15] (from 0.12 g of Mg) and 0.2 g of (I) in 1.5 ml of DMF was heated at 100° for 5 h, cooled, and diluted with ether. We obtained 0.85 g of the Mg salt (XIII). The treatment of 0.7 g of (XIII) with dilute HCl solution gave 0.3 g of (I).

Preparation of (XII) or N, N'-Dicarbomethoxy-(I). To a solution of t-C_4H_9OK (from 0.96 g of K and 30 ml of t-C_4H_9OH) was added 2 g of (I), and after 12 h was gradually added, with cooling in ice water and stirring, 2 ml of $ClCOOCH_3$, the filtrate was evaporated in vacuo, and the residue was recrystallized twice from water. We obtained 0.18 g (4%) of (XII), mp 236-237°, R_f 0.77 (acetone–water, 19.5 : 0.5). Found: C 41.35; H 5.02; N 14.28%. $C_7H_{10}O_5N_2$. Calculated: C 41.58; H 4.95; N 13.86%.

Carboxylation of Desthiobiotin (III). A mixture of (IV) (from 0.08 g of Mg) and 0.22 g of (III) in 1 ml of DMF was heated at 100° for 5 h, cooled, diluted with CH_3OH, and the obtained precipitate was centrifuged. We obtained 0.25 g of the Mg salt (XV) (white powder), which gave 0.1 g of (III) when treated with dilute HCl solution.

Carboxylation of Biotin (II). A mixture of (IV) (from 0.02 g of Mg) and 0.07 g of (II) in 1 ml of DMF was heated at 100° for 6 h, cooled, and diluted with 5 ml of CH_3OH. We obtained 0.1 g of the Mg salt (XIV) (white powder), which gave 0.04 g of (II) when treated with dilute HCl solution.

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CONCLUSIONS

1. The N-alkylation and N-carboxylation of the ambident salts of 2-imidazolidinone were accomplished in tert-butanol medium.

2. The N-carboxylation of 2-imidazolidinone, desthiobiotin, and biotin was run using magnesium methyl carbonate in dimethylformamide.

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