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Preliminary communication

THE SYNTHESIS OF ISOTOPICALLY LABELED AMINO ACIDS VIA COBALOXIME TEMPLATES

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Summary

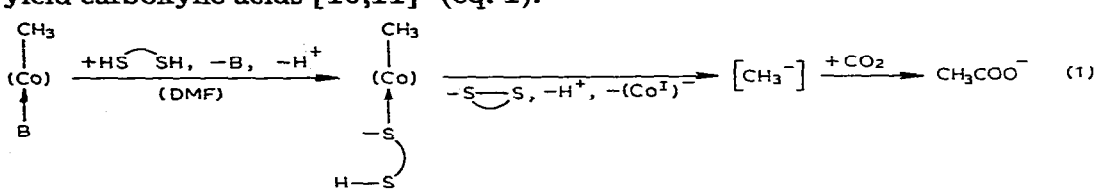
The synthesis of isotopically enriched amino acids employing a reduced form of bis(dimethylglyoximato) cobaltate (cobaloxime) as a reaction template is described. ^{13}C and/or ^{14}C enriched reagents ($*CO_2$ and H_2*CO) are used to achieve >90% isotopic enrichment at either or both of the carbon atoms in the glycinate portion of the amino acid chain.

The utility of isotopic enrichment for studies involving degradation or complexation reactions of amino acids or amino acid derivatives has been well documented [1—7].

Existing techniques for incorporating labeled carbon atoms into amino acids are generally quite laborious and time-consuming. Biological modes of synthesis utilize bacterial production of labeled species from enriched precursors. However, amino acids produced by such techniques rarely possesses isotopic enrichment at adjacent positions in the carbon chain.

In this communication we report a method for synthesizing ^{13}C or ^{14}C enrichment amino acids with isotopic enrichment occurring at either or both of the carbon atoms in the "glycinate" portion of amino acids.

It has been shown that an organocobaloxime with a $Co—CH_2—N$ linkage results when a mixture of formaldehyde and aniline is allowed to react with the reduced form of cobaloximes, (Co^I) [8—9]. Schrauzer and co-workers reported that methylcobaloximes undergo reductive cleavage in the presence of the reducing agent dithioerythyritol (DTE), to produce products which possess the reactivity of carbanions. These species in turn react with CO_2 to yield carboxylic acids [10,11] (eq. 1).

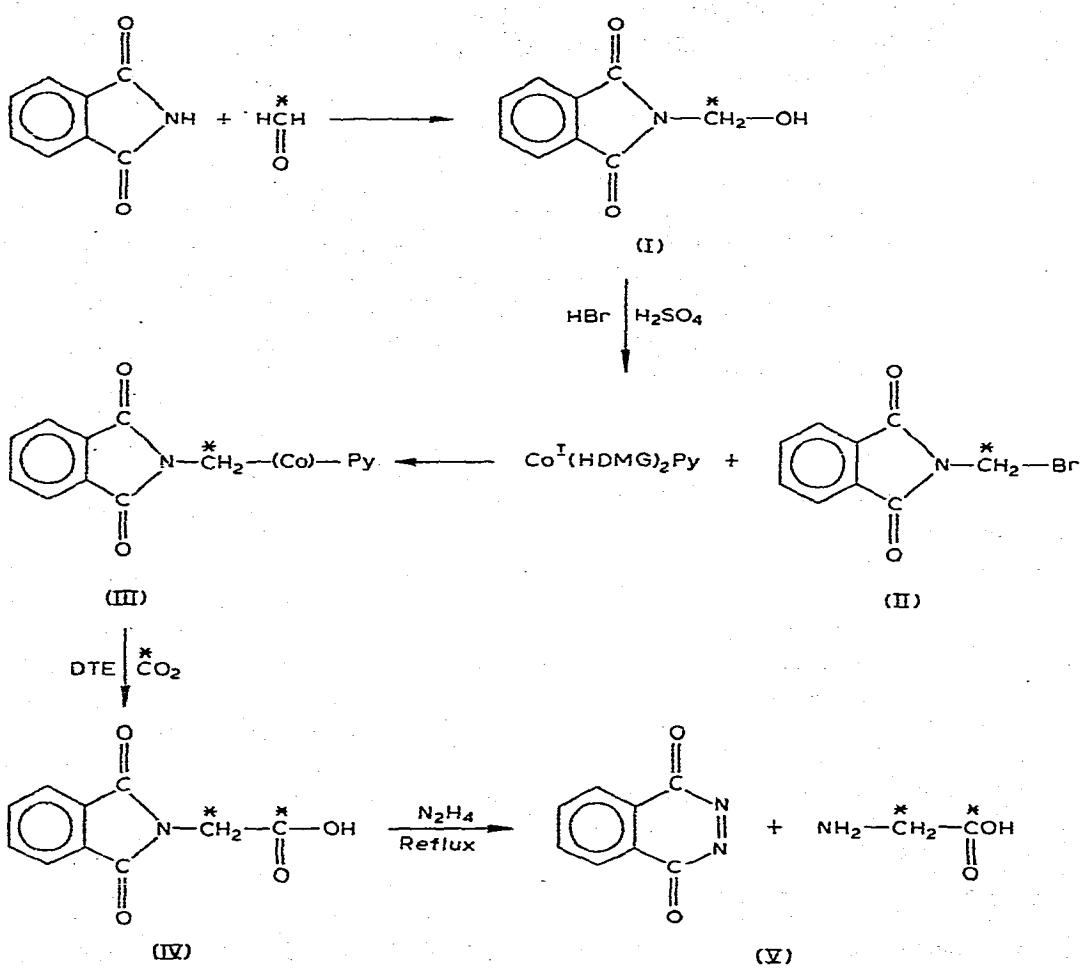


(1)

We employ a modified combination of some of the above reactions to synthesize amino acids with >90% enrichment at adjoining carbon atoms using relatively inexpensive isotopically enriched reagents, namely $*CO_2$ and H_2*CO. We have also synthesized some completely new organocobaloximes which yield amino acids with non-substituted amino groups (V below).

Due to its increased base strength over simple amines, and to its ability to release its nitrogen atom on treatment with hydrazine, phthalimide was chosen to react with labeled formaldehyde (Scheme 1).

SCHEME 1



Phthalimide, 1.5 g, was suspended in 2.1 ml of isotopically labeled (98% ^{13}C enriched) formaldehyde (38% solution) and 7 ml of H_2O. The suspension was refluxed at 98°C for 4 h to yield 1.4 g (80%) of *N*-(hydroxymethyl)-phthalimide (I). Compound I (1.0 g) was digested with 1.8 ml of 48% hydrobromic acid and 0.5 ml of concentrated sulfuric acid at 50°C for 2 h to yield 1.2 g (90%) of *N*-(bromomethyl)phthalimide (II). The *C enriched

bromide species II was then allowed to react with the reduced form of cobaloxime, (Co^{I}) (generated by the standard method described by Schrauzer [12]) to yield an extremely pure yellow-orange, crystalline, cobalt—carbon bonded product III (yield 86%). This species reacts with $^{*}CO_2$ (1 atm, in DMF), in the presence of the DTE reducing agent, to yield *N*-phthaloylglycine (IV) upon removal of the DMF solvent and extraction with benzene. The reaction was carried out by incubating the mixture at 60°C for 30 h (yield ~1%). Non-enriched IV, prepared by this method, exhibited identical thin layer chromatographic behavior and proton NMR spectra to that of authentic commercially prepared samples of the acid. Other selectively enriched amino acid derivatives, such as *N*-phenylglycine, may be prepared with equal ease by treating an aniline/formaldehyde mixture directly with the reduced form of cobaloxime (the intermediate bromide synthesis being omitted). The proton NMR spectrum of the anilinecobaloxime adduct, prepared with ^{13}C-labeled formaldehyde, is compared with that for the unlabeled adduct in Fig. 1. As

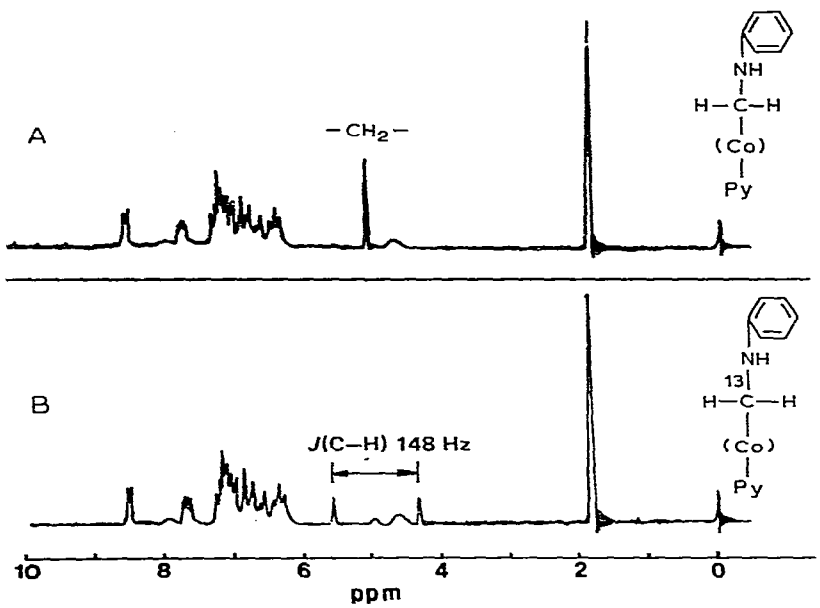


Fig. 1. 100 MHz proton NMR spectra of carbon-bonded *N*-methyleneanilinatopyridinatocobaloxime in $CDCl_3$ (A) prepared with normal formaldehyde (natural abundance in ^{13}C); (B) prepared with enriched formaldehyde (98% ^{13}C).

illustrated, the ^{13}C—H spin coupling (J(C—H) 148 Hz) observed is a useful diagnostic tool for assigning specific enrichment sites within a given molecule.

The phthaloyl derivative in Scheme 1 may be readily converted to the free amino acid. Hydrazine cleaves IV under aqueous reflux to yield V with isotopic enrichment at both carbons (yield >90%).

The utility of the labeling technique outlined above lies in the selectivity by which either or both of the carbon atoms in the amino acid chain may become isotopically enriched. Important applications include: (1) ^{13}C NMR studies for resonance line enhancements and ^{13}C spin—spin couplings, and (2) ^{14}C radio tracer studies involving biological samples.

Acknowledgements

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