reacted at -78 °C with lithium acetylide (100 mL of acetylene; *n*-BuLi, 1.0 mL, 1.5 M in hexanes; 15 mL THF) in the manner previously described.[17] After workup, the vacuum dried crude material (185 mg, ~0.63 mmol) in dry pyridine (5 mL) was treated with $POCl_3$ (1.0 mL, 93 μL/mmol, 10.8 mmol). After having refluxed the mixture for 6 h and then having stirred at room temperature for 12 h, standard workup afforded 103 mg (60% yield) of a mixture of enynes. The desired major enyne **23b** can be purified by reversed phase chromatography, but it is more efficient to couple this material via Scheme III and separate afterwards as described previously.[17]

Thermolysis of *cis*-Isotachysterols 1, 4, 7, and 10. Stereochemical and Kinetic Studies. Samples of the *cis*-isotachysterols were ascertained to be pure by HPLC (M-9 Partisil column, 3.0 mL/min flowrate, 10% EtOAc-SSB) and ^1H NMR and ^{13}C NMR spectra prior to the thermal investigations. The thermolysis solvent (spectral grade isooctane) was purified as previously described.[1] Isooctane stock solutions of **1**, **4**, **7**, and **10** were prepared under nitrogen at concentrations of ca. 2×10^{-3}–10^{-4} molar. From these stock solutions, 0.05-mL aliquots were taken and sealed under vacuum into 1 × 90 mm capillaries, which were stored at -80 °C until needed. The capillaries were fashioned from the narrow end of recleaned 9-in. disposable Pasteur pipets similar to that previously described.[1] For the kinetic studies, the sealed capillaries, after warming to ambient temperature, were placed in a thermostatically controlled oil bath (±0.05 °C) after temperature calibration. As the samples were removed after appropriate time intervals, the capillaries were stored at -80 °C until they were ready for analysis. Sample analyses were carried out by using HPLC with a UV detector set at 254 nm. The column was an analytical Waters μ-Porasil column (30 cm) with use of 1% isopropyl alcohol in Skellysolve B (SSB) as solvent and was calibrated with standard solutions of **1**–**6**. Table V in the Supplementary Material Section summarizes the data from 34 kinetic runs.

For the stereochemical studies, the product mixture from heating **7** (several capillaries were combined) for 26 h at 98.4 °C consisted of 22% **7**, 47% **9**, and 31% **8**; that from heating **10** under identical conditions consisted of 22% **10**, 15% **12**, and 63% **11**. Preparative separation was readily achieved by HPLC (Whatman M9 Partisil column, 10% EtOAc-hexanes). As summarized in Figure 1 and Table I, the deuterium analyses of the products **8**, **9**, **11**, and **12** were conveniently carried out by ^1H NMR integration of resonances assigned to H_1, $H_{6,7}$, H_{10}, and H_{15} of the products.

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Supplementary Material Available: Spectral and other experimental data (10 pages). Ordering information is given on any current masthead page.

^{13}C NMR Spectroscopy as a Biosynthetic Probe: The Biosynthesis of Purines in Yeast

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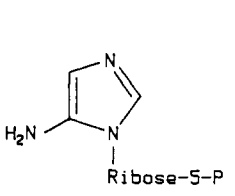
Abstract: It is shown by ^{13}C NMR spectroscopy that, in *Saccharomyces cerevisiae*, the skeleton of adenine and guanine arises from bicarbonate, which enters C-6, from formate, which enters C-2 and C-8, and from an intact glycine moiety, which gives rise to the C_2N unit, C-4, C-5, N-7. The findings substantiate general assumptions concerning the origin of the purines in yeast that had hitherto been based on fragmentary evidence. In *S. cerevisiae* the ring skeleton of the pyrimidine unit of vitamin B_1 (thiamin) and that of the purines do not originate from a common intermediate, unlike in bacteria where they do.

In *Salmonella typhimurium*,[1-5] and presumably in bacteria in general,[6] the pyrimidine unit **4** of thiamin is derived from 5-aminoimidazole ribonucleotide (AIR) (**1**), a compound that is an intermediate of purine biosynthesis.[7,8] Thus, in bacteria the biosynthesis of the thiamin pyrimidine unit is linked to that of the purines.

The pathway from AIR to the purines (e.g., guanine (**2**)) is well documented,[9,10] mainly on the basis of investigations with avian liver preparations. Individual steps of the pathway have been examined also in mammals (e.g., ref 11), bacteria (e.g., ref 12–15), and yeast,[14-17] and it is generally accepted[9,10] that the route from AIR to the purines is common to all organisms.



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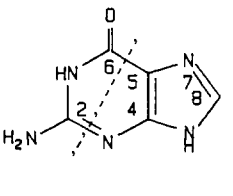

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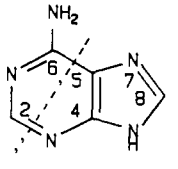

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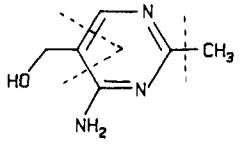

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The route to the thiamin pyrimidine unit **4**, on the other hand, is not identical in bacteria and in yeasts: In a recent study[18] of the biosynthesis of the pyrimidine unit of thiamin in *Saccharomyces cerevisiae* we investigated the distribution, within this unit, of label from [^{14}C]formate and from specifically [^{14}C]-labeled samples of glycine, glycerol, and glucose. From the observed distribution of label it must be concluded[18] that the biosynthesis of the pyrimidine unit of thiamin in this yeast, and possibly in yeasts in general,[6] differs substantially from its biosynthesis in bacteria.

The question of the relationship, in *S. cerevisiae*, of the biosynthetic route to the pyrimidine unit of thiamin with that leading to the purines, as well as the question of the identity of the basic building blocks of the purine skeleton in yeast, must therefore be re-examined. Re-investigation of the latter problem is necessary because, as will be documented below, existing evidence on the origin of the purines in yeast is fragmentary.

If the two ring systems **3** and **4** share a common origin in yeast, as they do in bacteria, i.e., if they are derived from a common intermediate, 5-aminoimidazole ribonucleotide (AIR) (**1**), then the pathway from primary precursors to AIR, i.e., the early steps of the biosynthetic route to the purines, cannot be the same in *S. cerevisiae* as the pathway to AIR in bacteria and in all other organisms in which it has been studied to date.[9,10]

If, on the other hand, the purine skeleton originates, in *S. cerevisiae* as it does in bacteria, from glycine, formate, bicarbonate, and ammonium ion, then, in yeast unlike in bacteria, the thiamin pyrimidine unit cannot share a common origin with the purines.

In order to answer these questions we have reinvestigated the incorporation of bicarbonate, formate, and glycine into purines, in cultures of *S. cerevisiae*, and have employed ^{13}C NMR spectroscopy to determine the distribution of ^{13}C within the samples of guanine (**2**) and adenine (**3**) isolated from these cultures after administration of ^{13}C-labeled samples of the C_1 substrates and of intramolecularly multiply ^{13}C–^{13}C and ^{13}C–^{15}N labeled samples of glycine.

Analysis of the spectra leads to the conclusion that in *S. cerevisiae* the purine bases are generated in the same manner in which they are derived in other organisms.[9,10]

It follows that, in *S. cerevisiae*, the carbon skeleton of the pyrimidine unit of thiamin and that of the purines do not originate from a common intermediate.

Results

Tracer experiments were carried out with 0.5-L cultures of *Saccharomyces cerevisiae* (A.T.C.C. 24903), growing on thiamin-free medium with glucose serving as the carbon source.[19] The ^{13}C-enriched substrate was added to the culture at the onset of logarithmic growth, and cells were collected after maximum growth had been attained. The purine fraction was isolated, and adenine and guanine were separated by cation exchange chromatography. The following ^{13}C-enriched compounds were administered in six individual tracer experiments: sodium [^{13}C]bicarbonate (99% ^{13}C, 0.4 g), sodium [^{13}C]formate (99% ^{13}C, 0.3 g), [1-^{13}C]glycine (99% ^{13}C, 0.2 g), [1,2-$^{13}C_2$]glycine (99% ^{13}C, 0.2 g), [2-^{13}C,^{15}N]glycine (99% ^{13}C, 99% ^{15}N, 0.2 g), and [2-^{13}C,^{15}N]glycine (99% ^{13}C, 99% ^{15}N, 0.2 g) in admixture with non-enriched sodium formate (0.6 g).

The ^{13}C NMR spectra of the samples of guanine (**2**) and adenine (**3**), obtained from these experiments, together with the natural abundance ^{13}C NMR spectra of the two bases, are presented in Figures 1 and 2, respectively.

The samples obtained from the experiment with [^{13}C]bicarbonate were enriched at C-6 (δ 153.8 and 151.8 respectively for guanine and adenine), the samples from the experiment with [^{13}C]formate were enriched at C-2 and C-8 (δ 155.8, 137.8 and 143.8, 145.5, respectively), and the samples from the experiment with [1-^{13}C]glycine were enriched at C-4 (δ 149.6 and 150.5, respectively).

When [2-^{13}C,^{15}N]glycine served as the substrate, enrichment was observed at three sites in each of the bases, C-5 (108.1 and 113.5 ppm respectively for guanine and adenine) as well as at C-2 and C-8, the sites that had shown enrichment with [^{13}C]formate.

When [2-^{13}C,^{15}N]glycine was administered in admixture with natural abundance formate, the signal due to C-5 again showed enrichment, but the signals due to C-2 and C-8 had almost disappeared.

The signal due to C-5 in these spectra appeared as a doublet ($^1J_{C\text{-}5,N\text{-}7}$ = 15.1 and 6.3 Hz respectively for guanine and adenine).

With [1,2-$^{13}C_2$]glycine as the substrate, the signals due to C-4 and C-5 (149.8, 107.5 and 150.2, 114.0 ppm respectively for guanine and adenine) showed enrichment and each appeared as a doublet ($^1J_{C\text{-}4,C\text{-}5}$ = 65.4 and 64.2 Hz, respectively). Enrichment was also detectable at C-2 and C-8.

Discussion

In the single reported investigation[20] of the incorporation, in *Saccharomyces cerevisiae*, of small molecules labeled with isotopic carbon into adenine and guanine, it was found that label from [^{14}C]formate and from [2-^{14}C]glycine entered the two bases.

Two degradation reactions were employed to determine the sites of labeling within the guanine samples: Hydrolysis with concentrated hydrochloric acid at 200 °C yielded glycine, which was assumed[21] to originate from C-4, C-5, and N-7 of the purine skeleton, on the basis of a 19th century investigation.[22] That the carboxyl carbon of the glycine originated from C-4 of guanine was shown[23] by degradation of a labeled sample of guanine, which was said to carry ^{13}C at C-4. The method of synthesis of this sample was not reported, however.[23]

The same degradation had been used earlier on a sample of guanine formed in another yeast, *Torulopsis utilis*, from [^{15}N]-glycine. Hydrolysis of this guanine sample yielded glycine enriched in ^{15}N, and it was concluded that precursor nitrogen had entered N-7 of guanine.[24] The danger of employing as a diagnostic indicator of a position of label in a metabolic end product a degradation product that is identical with the substance that had been used as a tracer is selfevident.

A similar degradation of guanine, high-temperature hydrolysis with concentrated sulfuric acid, is claimed, without experimental evidence, to yield C-2 plus C-6 as carbon dioxide, C-8 as carbon monoxide, and C-4,C-5 as glycine.[25]

In the second degradation method that was employed to locate label within guanine, the base was oxidized with acid permanganate, yielding guanidine, urea, and carbon dioxide. It was claimed,[21] without experimental evidence, that guanidine represents C-2, urea represents mainly C-8, and carbon dioxide was derived principally from C-4, -5, and -6.

On the basis of the labeling pattern of the products obtained by application of these degradation reactions, it was inferred[20] that, of the activity present in the guanine derived from [^{14}C]-formate in *S. cerevisiae*, 51% was located at C-2, 39% at C-8, and 15% at C-4,-5. Confidence limits for these incorporation data were not reported. These sites account for the total activity (105%) of the guanine sample. Similarly, the guanine derived from [2-^{14}C]glycine contained 22% of the label at C-2 and 54% at C-5.

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No radioactivity was found at C-4. Thus, 76% of the label of the intact molecule is accounted for. The remaining 24% must then be located at C-6 and/or C-8, sites that were not assayed.

No attempt was made to determine the distribution of label within the adenine isolated in these experiments with *S. cerevisiae*.[20]

These results constitute the entire evidence that is available on the distribution of label from carbon labeled substrates into purines formed in *S. cerevisiae*. It is therefore astonishing that statements such as the following are to be found in authoritative reviews:

"... The results of experiments in ... yeast ... demonstrate that glycine is the precursor of carbons 4 and 5 of the purine structure, whereas formic acid contributes to carbons 2 and 8 and carbon dioxide is the precursor of carbon 6. The general mechanism of synthesis of the purine skeleton is thus identical in the pigeon, rat, and yeast, regardless of the purine studied."[26]

"Thus, ... in yeast,[24] ... the incorporation of labeled precursors into adenine and guanine compounds occurs as anticipated ... ."[10]

More realistically, the following is stated in another review:[9] ... this appears to be the major route of synthesis of purines in avian liver. From a comparison of the number of intermediates or enzymes mediating their utilization which have been demonstrated in microbial species or mammalian and cancer tissues, it can be seen that our knowledge of the pathway in these other systems is somewhat limited however.".

In connection with our continuing investigation of the biosynthesis of vitamin B_1 in *S. cerevisiae* it was essential to answer in conclusive fashion the question of whether or not in this yeast the thiamin pyrimidine unit and the purines were of common origin. This was particularly important in view of the fact that while 5-aminoimidazole ribonucleotide (AIR) is formed in *S. cerevisiae*[27] and while the enzymic steps from 5-aminoimidazole ribonucleotide (AIR) to the purines have been investigated in *S. cerevisiae* and found to be identical with those occurring in other tissues,[14-16] the individual steps from primary precursors to AIR in this yeast are not documented.

The present work constitutes the first complete investigation of the origin, in *S. cerevisiae*, of the carbon skeleton of guanine (**2**) and adenine (**3**) from primary precursors.

The ^{13}C NMR spectra of the samples of guanine (**2**) (Figure 1) and adenine (**3**) (Figure 2), isolated from cultures of *S. cerevisiae* (A.T.C.C. 24903) that had been grown in the presence of ^{13}C-labeled substrates, show signals which indicate ^{13}C enrichment at the various carbon sites of the molecules. The sites of ^{13}C enrichment in these samples are readily deduced by comparison of their spectra with the ^{13}C NMR spectra of natural abundance samples of guanine and adenine.

These natural abundance ^{13}C spectra, determined under the same conditions as those of the enriched samples, in dimethyl sulfoxide solution containing concentrated hydrochloric acid, are shown in Figure 1A and Figure 2A, respectively. Me_2SO/H^+ was chosen as the solvent because these conditions afforded the best achievable resolution of signals of enriched carbon sites in the ^{13}C NMR spectrum of a crude culture extract containing a mixture of enriched bases (e.g., C-2 adenine 144, guanine 156; C-8 adenine 146, guanine 138 ppm). The spectrum of adenine, determined under these conditions, has been fully assigned.[28] The spectrum of guanine in Me_2SO/H^+ has not been reported. However, the spectrum of guanosine in acid (D_2O/DCl), which has been fully assigned,[29] shows signals due to the carbons of the heterocyclic system at chemical shifts corresponding to those now observed in the ^{13}C NMR spectrum of guanine in Me_2SO/H^+. In the absence of acid[30] the signal due to C-2 moves upfield and C-6

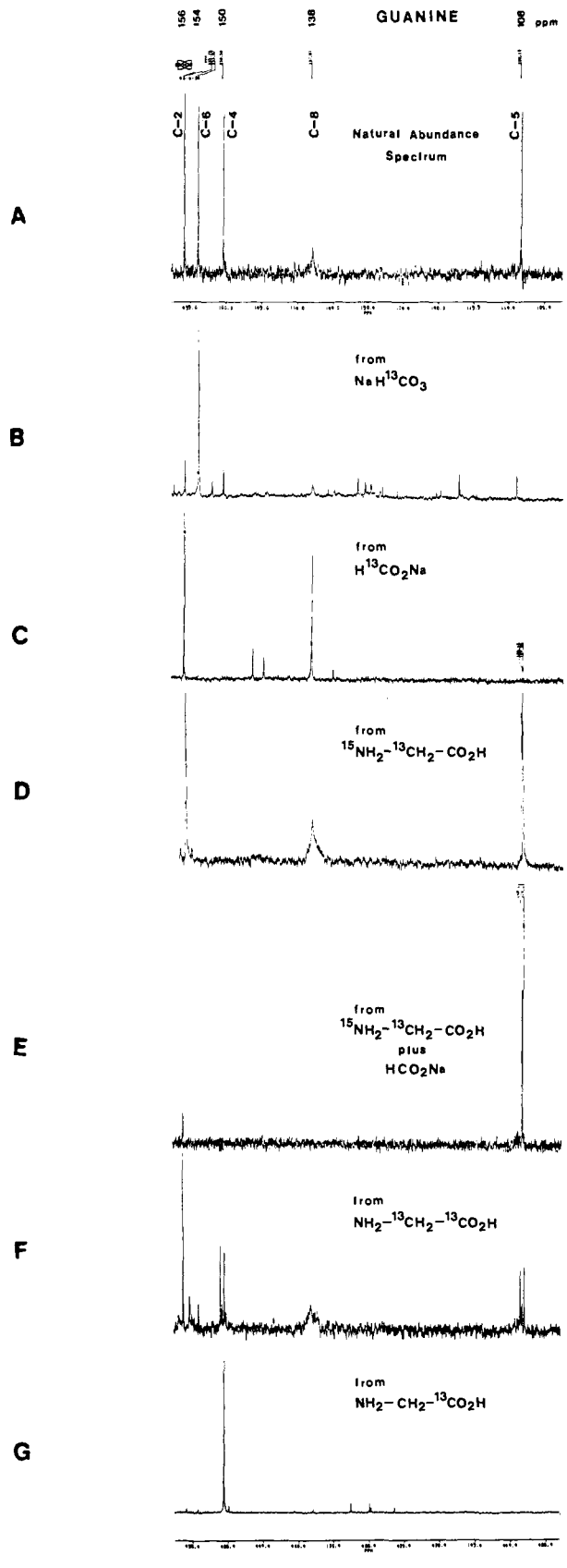

Figure 1. Composite pulse decoupled (CPD) 125.8-MHz ^{13}C NMR spectra of (A) a natural abundance ^{13}C sample of guanine and of (B–G) ^{13}C-enriched samples of guanine isolated from cultures of *S. cerevisiae* after incubation with the indicated ^{13}C-enriched substrates. The spectra were determined, on samples (<1 mg) in 5-mm tubes, in $[^2H_6]Me_2SO$ (250 μL) containing a drop of concentrated hydrochloric acid, on a Bruker AM 500 spectrometer.

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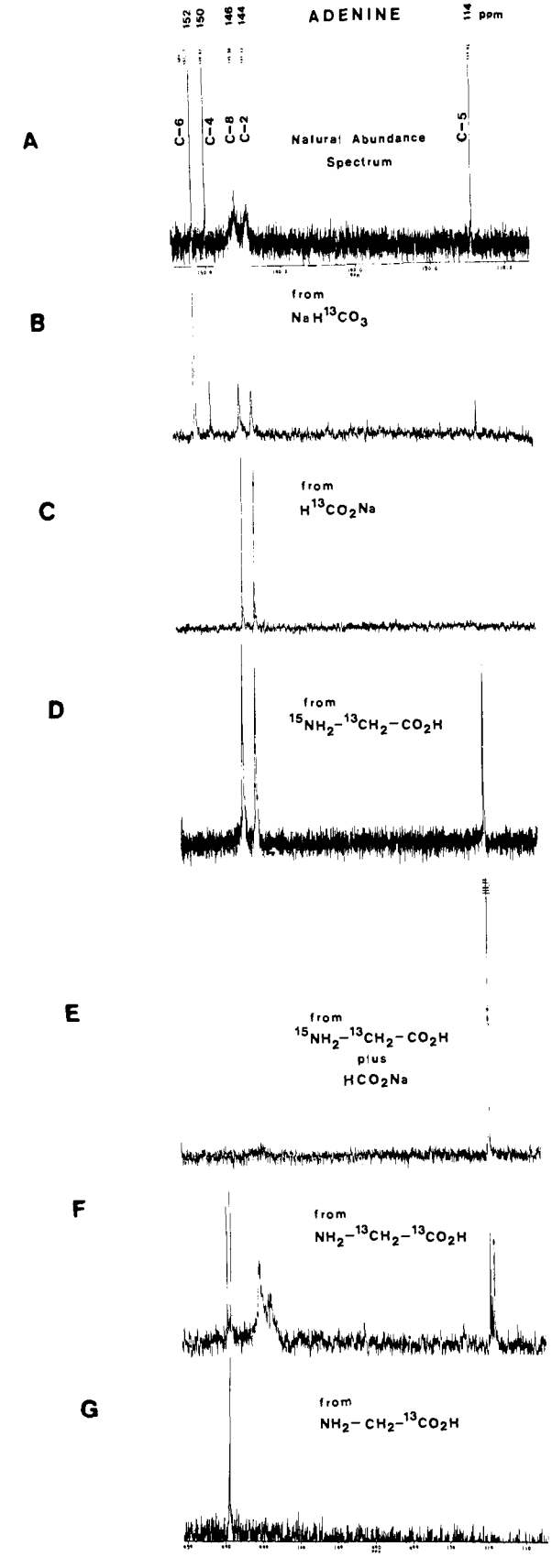

Figure 2. CPD 125.8 MHz ^{13}C NMR spectra of (A) a natural abundance ^{13}C sample of adenine and of (B–G) ^{13}C-enriched samples of adenine isolated from cultures of *S. cerevisiae* after incubation with the indicated ^{13}C-enriched substrates. The spectra were determined, on samples (<1 mg) in 5-mm tubes, in $[^2H_6]Me_2SO$ (250 μL) containing a drop of concentrated hydrochloric acid, on a Bruker AM 500 spectrometer.

yields the most downfield signal. The other signals maintain their relative positions.

Since it is generally accepted[9,10] that in mammals, birds, and bacteria the carbon atoms of the purine skeleton are derived from bicarbonate (C-6), formate (C-2 and C-8), and the carboxyl and methylene group of glycine (C-4 and C-5, respectively), these were the substrates that were tested as precursors of adenine and guanine in *S. cerevisiae*.

When [^{13}C]bicarbonate served as the substrate, the only carbon atom that was enriched, in the samples of guanine (**2**) (Figure 1B) and adenine (**3**) (Figure 2B) that were isolated, was C-6.

It has been inferred, on the basis of incomplete degradations of labeled samples, that bicarbonate[21,31] or carbon dioxide[32-34] serve as the source of C-6 of guanine in the intact rat[21] and in *E. coli*,[32] of C-6 of uric acid in the intact pigeon,[31,33,34] and of C-6 of caffeine in the coffee plant.[35] However, label from CO_2 was reported to enter not only into C-6 of uric acid (75% of the total label of the intact uric acid) but also into C-4 (20%).[31,33]

With [^{13}C]formate as the substrate, the samples of guanine and adenine showed enrichment at C-2 and C-8 (Figures 1C and 2C). In each case the two sites showed approximately equal enrichment and no other site was labeled.

In the only previous study of the incorporation of formate into guanine in *S. cerevisiae*,[20] the labeled sample was reported to contain approximately 51% of its label at C-2, approximately 39% at C-8, and approximately 15% at C-4,-5.

Label from formic acid has been observed to enter C-2 and C-8 of guanine and adenine in the intact rat[21,36] and the intact chicken,[25] C-2 and C-8 of uric acid in the intact pigeon,[31,37,38] and C-2 and C-8 of caffeine in coffee.[35] In the former cases[21,25,36,38] entry of label into C-2 and C-8 was approximately equal. In the latter case,[35] incorporation of label into C-2 was 3–4 times more efficient than incorporation into C-8.

By contrast, in bacteria label from formate enters C-8 much more readily than C-2. Thus, in *Pseudomonas fluorescens* and *Aerobacter aerogenes*, formate activity labeled C-8 but not C-2 of adenine and guanine, and most of the formate-derived label was located elsewhere in the molecules, presumably at C-6.[39] Similarly, in *E. coli* significantly more label from formate entered C-8 than C-2 of adenine (ca. 11:1).[40,41] In yet another bacterium, *Mycobacterium tuberculosis*, formate did not appear to be an important purine precursor.[42] Nor did formate serve as a precursor of the adenine moiety of aristeromycin in *Streptomyces citricolor*.[43]

In all these bacteria, the α-carbon atom of glycine served as the most important source of C-2 and C-8 of the purine skeleton.

Label from the methylene carbon of glycine enters C-5, as well as C-2 and C-8 of guanine (Figure 1D) and adenine (Figure 2D) when [2-^{13}C,^{15}N]glycine was the only substance added to the glucose-based thiamin-free culture medium. In order to establish whether the methylene group of glycine entered the formate-derived carbons (see Figures 1C and 2C) directly or after conversion to formate, a culture was incubated with the same substrate, [2-^{13}C,^{15}N]glycine, but with simultaneous addition of excess natural abundance formate. The samples of guanine (Figure 1E) and adenine (Figure 2E) isolated from this experiment were

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enriched at C-5 as before, but, unlike in the preceding experiment, C-2 and C-8 were not enriched. This indicates that the methylene carbon of glycine enters C-5 of the purine system as such but that its entry into C-2 and C-8 takes place only after conversion into formate.

In both experiments with [2-^{13}C,^{15}N]glycine, the signal due to C-5 appears as a doublet in the spectrum of guanine ($^1J_{C\text{-}5,N\text{-}7}$ = 15.1 Hz, Figure 1, parts D and E) and adenine ($^1J_{C\text{-}5,N\text{-}7}$ = 6.3 Hz, Figure 2, parts D and E), due to ^{13}C,^{15}N coupling of C-5 with the adjacent ^{15}N-enriched site N-7. This constitutes direct proof for the intact incorporation into the purine skeleton of a C–N unit derived directly from CH_2NH_2 of glycine.

Intact incorporation of a C–N unit derived from (2-^{13}C,^{15}N)glycine was not observed in a recent ^{13}C NMR study of the origin of the adenine moiety of the antibiotic aristeromycin.[43]

An experiment with [1,2-$^{13}C_2$]glycine as the substrate demonstrates intact incorporation into C-4,-5 of the purine skeleton of a C–C unit derived directly from HO_2C–CH_2 of glycine.

The spectra of the samples of guanine (Figure 1F) and adenine (Figure 2F) isolated from this experiment show signals due to enrichment at C-5 (107.5 and 114.0 ppm, respectively) and C-4 (149.8 and 150.2 ppm, respectively). The two carbon atoms are coupled since their signals appear as doublets ($^1J_{C\text{-}4,C\text{-}5}$ = 65.4 Hz for guanine and 64.2 Hz for adenine).

The experiment with [1,2-$^{13}C_2$]glycine was carried out without addition of formate, and enrichment at C-2 and C-8, due to entry into these two sites of label from the methylene group of glycine, via formate, is discernible in the spectra of guanine (Figure 1F) and adenine (Figure 2F).

The final experiment, with [1-^{13}C] glycine, yielded samples of the two bases showing enrichment solely at C-4 (Figures 1G and 2G).

The experiments with [2-^{13}C,^{15}N]glycine and with [1,2-$^{13}C_2$]glycine together constitute the first tracer experiments whose results offer direct proof for the intact incorporation of a C–C–N unit, derived from glycine, into C-4,C-5,N-7 of the purine skeleton of adenine and guanine.

Earlier tracer work with ^{13}C and ^{15}N employing mass spectrometry to determine isotope enrichment, and with ^{14}C, led to the suggestion that the C_2N unit, C-4,C-5,N-7 of the purines, was derived as a unit from glycine. The methods that were employed could not provide unequivocal proof for direct entry of a glycine-derived C–C–N unit into the purine skeleton. Participation in purine biosynthesis of glycine as a unit was demonstrated when the enzymic steps of the pathway were established.[44,45]

Heavy nitrogen from [^{15}N]glycine was reported to enter N-7 of uric acid in man,[46] of hypoxanthine in pigeon tissue,[47] and of guanine in a yeast, *Torulopsis utilis*.[24] Label from [1-^{13}C]-glycine[33,34] and [1-^{14}C]glycine[21,31] has been reported to enter mainly C-4 of guanine in the rat[21] and of uric acid in intact pigeons.[31,33,34] Label from [2-^{14}C]glycine was reported to reside not only at C-5 of uric acid from intact pigeons (52%) but also at C-4 (15%) and at C-2 plus C-8 (31%).[31] Similarly, label from [2-^{14}C]glycine in guanine from *S. cerevisiae*[20] was located at C-5 (54% of total activity of intact guanine), C-2 (22%), and C-8 (and/or C-6) (24%, by difference).

In *Pseudomonas fluorescens*, *Aerobacter aerogenes*, *Escherichia coli*,[40,48] and *Mycobacterium tuberculosis*[42] label from [2-^{14}C]-glycine enters C-2 and C-8 of adenine[39,40,42] and guanine,[39,42] as well as other (unidentified) sites. With increasing glycine concentration in the medium, label from [2-^{14}C]glycine enters progressively into C-5, C-8, and C-2 of the purines in *E. coli*.[48]

Very recently, in a ^{13}C NMR study of the origin of the adenine moiety of the antibiotic aristeromycin produced by *Streptomyces citricolor*,[43] a C_2 unit from [1,2-$^{13}C_2$]glycine was shown to enter C-4,C-5 of the antibiotic directly, as indicated by coupling between C-4 and C-5 ($^1J_{C\text{-}4,C\text{-}5}$ = 65 Hz). Furthermore, ^{13}C from [2-^{13}C,^{15}N]glycine entered C-5. In addition, C-2 and C-8 of the purine were highly enriched, in each case.

It would appear that, in bacterial systems in general, the methylene group of glycine is a better source of C-2 and C-8 of the purine nucleus than is exogenously supplied formate. Possible reasons for this observation have been discussed.[39,40,42]

Textbooks and reviews convey the impression that the process of purine biosynthesis follows the same pattern in all species. While this is clearly not so and a conscientious reading of the literature reveals significant species variations, it would appear that the basic building blocks of the purine skeleton, bicarbonate, formate, glycine, and ammonia, are similar in all species that have been studied and species variations of incorporation patterns are the result of secondary processes.

We have now shown that the origin of the purine skeleton, as exemplified by adenine and guanine, is indeed similar in *S. cerevisiae* and in pigeons, the biological system which was used in the classical studies of purine biosynthesis some 40 years ago.

Since, as we have now demonstrated, the purines of *S. cerevisiae* are derived in the classical manner, it follows from our earlier investigation[18] of the biosynthesis of thiamin (vitamin B_1) that in *S. cerevisiae* the pyrimidine unit of vitamin B_1 does not share a common origin with the purines. The derivation of the thiamin pyrimidine thus demands renewed attention.

Experimental Section

Microorganism. *Saccharomyces cerevisiae* (A.T.C.C. 24903) was maintained on malt extract/yeast extract/peptone/glucose slants as described previously.[19]

Medium. The glucose-based thiamin-free medium, previously described,[19] was used.

Procedures for Growth and Administration of ^{13}C-Enriched Substrates. The procedures employed in experiments with glucose-based medium have been reported.[19]

Isolation of Purines (cf. ref 49). In each experiment, cells from a 0.5-L culture were separated by centrifugation. The cells were suspended in dilute hydrochloric acid (0.3 M, 20 mL) and were lysed by warming the suspension 1 h on the steam bath. The cell debris was centrifuged off and re-extracted with dilute hydrochloric acid (0.3 M, 20 mL) for 1 h on the steam bath. The combined solution was evaporated in vacuo, the residue dissolved in water (5 mL), and the solution applied to a column of Dowex 50 W × 4 (100–200 mesh, H^+ cycle, 20 g). Guanine (80–130 mL eluate) and adenine (150–230 mL eluate) were eluted with a solution of potassium dihydrogen phosphate (0.5 M, pH 4.3).

The fractions containing guanine and adenine, respectively, were evaporated and the residue from each was suspended in dimethyl sulfoxide (5 mL) containing 2 drops of concentrated hydrochloric acid. After 12 h each suspension was filtered to remove inorganic salts, and the filtrates were evaporated. The salts of guanine and adenine, respectively, which were obtained in this way were redissolved in [$^2H_6$]dimethyl sulfoxide (0.5 mL) containing a drop of concentrated hydrochloric acid. These solutions were used to determine the ^{13}C NMR spectra of the samples.

^{13}C NMR Spectra. Spectra were determined at 125.8 MHz on a Bruker AM 500 spectrometer under standard conditions. The spectra are presented in Figures 1 and 2.

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