DL-α-**Lipoic Acid-$S_2^{35}$ (IV).**—To a 15 × 180 mm. tube, equipped with a Hershberg-type stirrer and immersed in a Dry Ice–isopropyl alcohol mixture, was added in succession 5 ml. of anhydrous liquid ammonia, 16 mg. of sodium wire, and a solution of 50 mg. (0.13 mmole) of DL-6,8-dibenzylmercaptoöctanoic acid-$S_2^{35}$ in 2 ml. of peroxide-free, anhydrous ether. The mixture was stirred until decolorized and small pieces of sodium wire were added until a permanent blue color resulted. The blue color was discharged with ammonium chloride, the cooling bath was removed, and the liquid ammonia and ether were allowed to evaporate under a slow stream of nitrogen. To the residue was added 4 ml. of water and the *p*H was adjusted to 9 with 2 *N* hydrochloric acid. Two drops of 1% ferric chloride solution were added and a stream of air was bubbled through the solution from a capillary tube until the reddish color changed to pale yellow (approximately 15 minutes). The mixture was extracted with two 2-ml. portions of peroxide-free ether to remove some suspended solid and then acidified with 6 *N* hydrochloric acid. The product was extracted with three 1-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and then carefully evaporated in a small sublimation apparatus with a stream of nitrogen. The last traces of solvents were removed at $10^{-4}$ mm. The solid residue of crude DL-α-lipoic acid-$S_2^{35}$ was distilled[11] at $10^{-4}$ mm. and 90° onto the cold finger which was cooled with powdered Dry Ice. The solid product adhering to the cold finger was rinsed with anhydrous benzene into a small weighing tube. The solvent was removed with a slow stream of dry nitrogen and finally *in vacuo* (oil pump). The yield of microcrystalline product was 19.5–22.8 mg. (73–86%), m.p.[10] 60.5–61.5° (uncor.), $\epsilon_{max}$ 147 (332 mμ), specific activity 68 μc./mg.

(11) In trial runs with non-radioactive materials attempts to crystallize the crude product from Skellysolve B[3] produced varying amounts of a Skellysolve B-insoluble viscous material and, consequently, low yields of crystalline product. However, when the crude product was distilled as described, only traces of residue remained.

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## Biosynthesis of Phenylalanine in Bakers' Yeast[1,2]

BY C. R. THOMAS[3], B. E. CHRISTENSEN, V. H. CHELDELIN AND C. H. WANG

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In contrast to the numerous reports on the biosynthesis of tyrosine in microörganisms, the analogous formation of phenylalanine has not been thoroughly investigated. Davis has shown that in *Escherichia coli*, phenylalanine is not a normal precursor of tyrosine.[4] This supports the observation of Simmonds, *et al.*,[5] that tyrosine does not exert a sparing action on the quantitative requirement of a phenylalanine requiring mutant strain of *E. coli*.

On the other hand, the accumulation of prephenic acid by certain tyrosine and phenylalanine auxotrophs[4] points to a possible common biosynthetic pathway leading to these two amino acids. Studies carried out by Gilvarg and Bloch[6] with yeast grown on glucose-1-$C^{14}$ revealed a close resemblance of the labeling pattern of phenylalanine to that of tyrosine from the same yeast. This also suggested that these two amino acids may have been synthesized from glucose by the same general mechanism.

In this Laboratory, isotopic distribution patterns of phenylalanine have been obtained from yeast[7,8] grown on $CH_3C^{14}OCOOH$ and $CH_3C^{14}OOH$. Comparison of these patterns with those in tyrosine isolated from the same yeast[9] have revealed that whereas from pyruvate the isotopic distributions are practically identical, sharp divergences occur when acetate is the substrate. Thus, from acetate, carbons 2 + 4 + 6 of the tyrosine ring account for over 70% of the ring activity,[9] while in phenylalanine the bulk of the ring activity is located in carbons 1 + 3 + 5.

### Experimental

Use was made of the yeast samples grown on $CH_3C^{14}OCOOH$ and $CH_3C^{14}OOH$ as described previously.[7]

After microbiological assay of the phenylalanine in the neutral amino acid fraction of the defatted yeast hydrolysate,[8] this amino acid was diluted ninefold with inert (carrier) phenylalanine. The diluted sample was separated from other amino acids by Dowex-50 column chromatography, using a modified version of the method of Stein and Moore.[10] Isolation was completed by the method of Cutinelli, *et al.*[11] The identity and purity of the final product were established by paper chromatography. The quantities of diluted phenylalanine obtained in this manner from three yeast samples were: from acetate, 244 mg.; from pyruvate (aerobic), 337 mg.; and from pyruvate (anaerobic), 284 mg.

The phenylalanine samples thus obtained were degraded according to the method of Gilvarg and Bloch.[6] The results of these degradation studies on phenylalanine are given in Table I, with the earlier data on tyrosine[9] included for comparison. All samples were counted as $BaCO_3$, using a windowless gas-flow Geiger–Müller counter. Counting data were corrected for background and self-absorption in the conventional manner. Since the previous observations on tyrosine were made with the use of a mica end-window counter, the present figures have been reduced by an appropriate factor to render the phenylalanine and tyrosine data comparable. All data are given for the undiluted amino acids.

### Discussion

The labeling patterns of the *side chains* of phenylalanine (Table I) from either acetate or pyruvate are in good agreement with those reported in the corresponding tyrosine experiments,[9] hence give additional support to the suggestion[6,9,12] that the side chains of both amino acids originate from an intact $C_3$ unit such as pyruvate. The mechanism is also in line with the recent findings,[13,14] that prephenic acid, formed by condensation of shikimic

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(2) Taken in part from the Ph.D. thesis of R. C. T., Oregon State College, 1954.

(3) National Science Foundation Predoctoral Fellow.

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TABLE I

DISTRIBUTION OF $C^{14}$ IN PHENYLALANINE AND TYROSINE FROM YEAST UTILIZING $CH_3-C^{14}O-COOH$ OR $CH_3-C^{14}OOH$

| | Phenylalanine | | | | | | Tyrosine | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aerobic pyruvate | | Anaerobic pyruvate | | Aerobic acetate | | Aerobic pyruvate | | Anaerobic pyruvate | | Aerobic acetate | |
| Conditions → | C.p.m./ mmole × $10^4$ | % of total | C.p.m./ mmole × $10^4$ | % of total | C.p.m./ mmole × $10^4$ | % of total | C.p.m./ mmole × $10^4$ | % of total | C.p.m./ mmole × $10^4$ | % of total | C.p.m./ mmole × $10^4$ | % of total |
| Whole molecule | 38.8 | 100 | 19.0 | 100 | 4.58 | 100 | 49.1 | 100 | 19.0 | 100 | 4.83 | 100 |
| Side chain | 18.4 | 47.3 | 10.0 | 52.7 | 1.33 | 29 | 22.3 | 48 | 7.7 | 41 | 2.43 | 50 |
| Ring | 20.4 | 52.7 | 9.0 | 47.3 | 3.25 | 71 | 25.9 | 53 | 10.5 | 55 | 2.59 | 53 |
| Distribution of $C^{14}$ within the side chain | | | | | | | | | | | | |
| $CH_2CHNH_2COOH$ | 18.4 | 100 | 10.0 | 100 | 1.33 | 100 | 22.3 | 100 | 7.7 | 100 | 2.43 | 100 |
| COOH | 2.7 | 14.7 | 2.7 | 27 | 1.33 | 100 | 4.3 | 19 | 2.8 | 36 | 2.43 | 100 |
| $CHNH_2$ | 14.8 | 80.3 | 6.5 | 65 | 0 | 0 | 18.0 | 81 | 4.9 | 64 | 0 | 0 |
| $CH_2$ | 0.9 | 5 | 0.8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Distribution of $C^{14}$ within the ring | | | | | | | | | | | | |
| Ring | 20.4 | 100 | 9.0 | 100 | 3.25 | 100 | 25.9 | 100 | 10.5 | 100 | 2.59 | 100 |
| $C_{1+3+5}$ | 16.5 | 81 | 6.0 | 66.6 | 2.50 | 77 | 20.1 | 78 | 5.4 | 52 | 0.74 | 28 |
| $C_{2+4+6}$ | 3.9 | 19 | 3.0 | 33.3 | 0.75 | 23 | 5.7 | 22 | 5.0 | 48 | 1.74 | 72 |
| $C_1$ | | | | | | | 8.3 | 32 | 1.6 | 15 | 0 | 0 |
| $C_{3+5}$ | | | | | | | 11.8 | 46 | 3.0 | 36 | 0.74 | 29 |
| $C_4$ | 1.7 | 8.3 | 1.4 | 15.6 | 0.58 | 17.6 | | | | | | |
| $C_{2+6}$ | 2.2 | 10.8 | 1.6 | 17.8 | 0.17 | 5.1 | | | | | | |

acid and a $C_3$ unit, is an intermediate in the biosynthesis of phenylalanine.

Although the precise mechanism of formation of phenylalanine in yeast is conjectural, the present data permit some pertinent conclusions regarding the process. First, tyrosine is not directly interconvertible with phenylalanine, at least in the acetate samples. Second, whereas from pyruvate or glucose a single route of synthesis of phenylalanine and tyrosine may function,[4,6] with acetate as substrate there are two important differences: the orientation of the side chain in terms of isotopic distribution in the ring, and the unequal distributions of isotope between the rings and the side chains (Table I). The different orientations of the side chains might be either the result of a change in the site of attachment, or a migration of the side chains after attachment.

The picture is further complicated by the differing isotope contributions of the ring and side chain. With *pyruvate* as the carbon source, the labeling in the two amino acids is similar in amount and almost identical in pattern (Table I), but with acetate as substrate, phenylalanine is preferentially labeled in the ring. It would appear from these observations that the routes of synthesis of the *aromatic ring* may also differ in one or more important respects when derived from acetate or pyruvate.

Acetate, although not a "normal" substrate for yeast, has been widely used to suggest pathways of amino acid biosynthesis. In view of the present results, a note of caution seems appropriate when observations on acetate metabolism are interpreted in terms of pyruvate, glucose, or other carbohydrate intermediates.

Efforts are continuing to outline the routes of biosynthesis of these amino acids.

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## Methyl D-Glucoside Tetra-(chlorocarbanilates)

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In previous studies at this Laboratory[1] it was shown that tri-*o*-chlorocarbanilates of corn starch and its fractions had solubility properties, optical rotations and melting ranges considerably different from the corresponding *meta* isomers. This was attributed to the existence of a chelate structure in the *ortho* compounds. It also was pointed out that the observed properties might result from the additive effect of the small individual contribution of each chelated chlorine atom caused by the large total number of such groupings in the polymer molecules. To investigate this point further, it was considered desirable to compare the properties of isomeric chlorocarbanilates of non-polymeric materials. The anomeric methyl D-glucosides were selected for derivatization and study.

### Experimental

**Materials.**—Eastman Kodak Co. white label isocyanates were used as received without further purification. They were characterized by conversion in high yield to the corresponding ureas which had melting points corresponding to literature values. Anomeric methyl D-glucosides were prepared by conventional procedures and dried before use.

**Preparations.**—Only minor variation of the following general preparative procedure was employed for the individual compounds. To 1.94 g. (10 millimoles) of the glucoside in 15 ml. of dry pyridine was added 1.1 to 1.2 times the theoretical amount of isocyanate required for complete substitution. All reactions were exothermic. After allowing the reaction mixtures to stand overnight at room temperature 10–15 ml. of dry methanol was added, followed, after about an hour, by 25–50 ml. of water. Total yields of crude carbanilates were usually slightly above theoretical due to contamination with by-products from the excess reagent used. Preferred solvent systems for purification of the products, together with properties of the compounds, are listed in Table I.

Similar to the glycoside carbanilates described by Wol-

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