

ternatively the epoxide could isomerize to dienols through the  $\pi$ -allyl complex, **9** or **10**. The detailed mechanistic study is now in progress.

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- (6) Pd(PPh<sub>3</sub>)<sub>4</sub> was the best catalyst for the isomerization, 1 → 2. Other catalysts examined were Pd(acac)<sub>2</sub>-PPh<sub>3</sub> (11 mol %, benzene, 50 °C, 11 h, 53%), PdCl<sub>2</sub>-PPh<sub>3</sub> (13 mol %, THF, 50 °C, 59 h, 35%), Pd<sub>2</sub>(tribenzylideneacetone)<sub>2</sub>-CHCl<sub>3</sub> (supplied kindly by Professor K. Itoh)-PPh<sub>3</sub> (3.4 mol %, benzene, 50 °C, 11 h, 65%), Pt(PPh<sub>3</sub>)<sub>4</sub>-PPh<sub>3</sub> (3.9 mol %, 25 °C, 50 h, 39%), MgBr<sub>2</sub> (9 mol %, ether, 50 °C, 11 h, 29%), LiClO<sub>4</sub>·3H<sub>2</sub>O (11 mol %, ether, 50 °C, 11 h, 33%), LiBr·H<sub>2</sub>O (37 mol %, ether, 50 °C, 23 h, 14%). The following compounds were inactive for the isomerization under comparable reaction conditions: Pd<sub>2</sub>(tribenzylideneacetone)<sub>2</sub>-CHCl<sub>3</sub>, Pd(PhCN)<sub>2</sub>Cl<sub>2</sub>, Na<sub>2</sub>PdCl<sub>4</sub>, Pd/C, Rh(PPh<sub>3</sub>)<sub>3</sub>Cl, Ni(PPh<sub>3</sub>)<sub>2</sub>(CO)<sub>2</sub>-PPh<sub>3</sub>, Ni(acac)<sub>2</sub>.
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## New Nuclear Magnetic Resonance Methodology in Biosynthetic Studies of Mixtures of Statistically Enriched <sup>13</sup>C and Unlabeled Precursors

Sir:

An increasing number of analyses of biosynthetic experiments using double labeling has been reported either with two <sup>13</sup>C carbon atoms<sup>1</sup> or with one <sup>13</sup>C and one <sup>2</sup>H.<sup>2</sup> However, reports on experiments carried out with statistically enriched precursors are sparse, especially in the field of polysaccharides. It is clear that NMR analyses of spectra of biosynthesized compounds can give unique information on the origin of groups of coupled carbon atoms, since complex mixtures of isotopomers can be analyzed from <sup>13</sup>C experimental spectra. In some particular cases a more powerful technique, providing both structural and biochemical informations, is obtained by using a mixed precursor of unlabeled material and statistically <sup>13</sup>C-enriched material.

The basic principle can be illustrated with a very simple

example: the final product has two adjacent carbons, C-1 and C-2 for instance, with the same fraction ( $\tau$ ) of labeling with <sup>13</sup>C and is supposed to be formed in 100% yield from a 1:1 mixture of unlabeled and statistically enriched <sup>13</sup>C precursor. If we define  $P_{12}$  as the probability of both carbons in the final product coming from the same precursor molecule of either the unlabeled or statistically enriched fractions, and  $P_{1/2} = 1 - P_{12}$  as the probability of their coming from different precursor molecules, the four populations of isotopomers having <sup>12</sup>C or <sup>13</sup>C at C-1 or C-2 are only a function of  $P_{12}$ . In particular, if we consider the <sup>13</sup>C-1 spectrum, we see that this carbon is either coupled or noncoupled to the neighboring C-2. If we call  $X_{12}$  the probability of <sup>13</sup>C-1 having a <sup>13</sup>C-2 as a neighbor, we have

$$X_{12} = P_{12}[\alpha\rho_A^2 + (1 - \alpha)\rho_B^2]/\tau + [1 - P_{12}]\tau$$

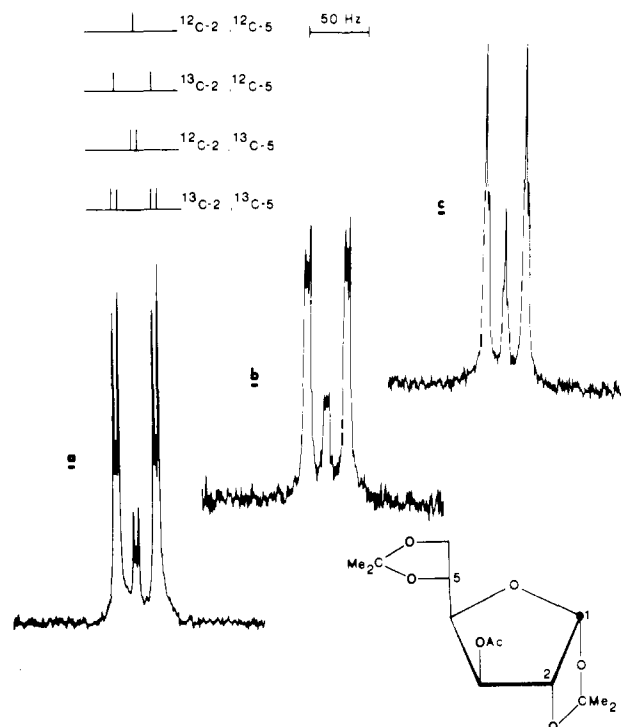
The first term refers to two <sup>13</sup>C atoms coming without cleavage from the precursor of either the unlabeled or statistically enriched fractions; the second is related to two <sup>13</sup>C atoms coming from two different fragments cleaved and recombined. In this expression,  $\rho_A$  is the fraction of <sup>13</sup>C in the statistically enriched precursor;  $\rho_B$  is the fraction of <sup>13</sup>C in the "unlabeled" precursor ( $\rho_B = 0.01$ , but to a first approximation  $\rho_B = 0$  can be used);  $\alpha$  is the fraction of the statistically enriched precursor incorporated in the final product at positions C-1 and C-2—this value has to be determined experimentally by means of the relation  $\tau = \alpha\rho_A + (1 - \alpha)\rho_B$  and is generally smaller than the fraction of labeled precursor used. The general derivation of the expression proceeds with consideration of the four isotopomers having <sup>12</sup>C or <sup>13</sup>C at C-1 and C-2. Experimental determination of  $X_{12}$  gives then  $P_{12}$ , which value is a number between 0 (all groups of C-1 and C-2 atoms come from different precursor molecules) and 1 (all groups of C-1 and C-2 atoms come from the same precursor molecule).

The mathematical treatment proposed here, more general than that given by Tran-Dinh et al.<sup>3</sup> and by London et al.,<sup>1h</sup> can then be used with any groups of more than two atoms. For a group of three consecutive atoms such as C-1, C-2, and C-3, the observation of the spectrum for the "middle" atom (in this case C-2) provides experimental data about the four species corresponding to <sup>12</sup>C or <sup>13</sup>C at C-1 or C-3. Thus we can define four probabilities resulting from the four possible origins of the three carbon atoms (i.e., from the same precursor molecule, from two molecules (two cases), and from three different molecules). This gives three independent parameters or one new correlation term  $C_{13}$  if  $P_{12}$  and  $P_{23}$  are known. The definition (and sign) of this correlation term can be determined from one of the following equations:  $P_{123} = P_{12}P_{23} + C_{13}$  (in the case of the three atoms originating from the same precursor molecule of either the unlabeled or statistically enriched fraction);  $P_{1/23} = (1 - P_{12})P_{23} - C_{13}$  (C-1 comes from one molecule, C-2 and C-3 from another);  $P_{12/3} = P_{12}(1 - P_{23}) - C_{13}$  (C-1 and C-2 come from one molecule, C-3 from another);  $P_{1/2/3} = (1 - P_{12})(1 - P_{23}) + C_{13}$  (three atoms from three different precursor molecules).

More detailed calculations and generalization to a group of four atoms are given elsewhere.<sup>4</sup> In this case two new correlation terms are necessary, but experimental data to date are perfectly described by only  $P$  and  $C$  values.

As a typical example, biosynthesis of cellulose with *Acetobacter xylinum*, as described by Hestrin,<sup>5</sup> has been chosen, since it is a rather complicated in vivo biosynthesis with at least three principal pathways.<sup>6</sup> It is also a case where many experiments of labeling with <sup>14</sup>C,<sup>6,7</sup> <sup>2</sup>H,<sup>8</sup> and <sup>13</sup>C<sup>4</sup> have been performed, particularly in our laboratory, thus giving a clear indication of the significance of the methodology proposed here.

The NMR analysis, for the starting material<sup>9</sup> and for the biosynthesized cellulose, has been performed with chemical



**Figure 1.** The proton decoupled  $^{13}\text{C}$  NMR spectra (62.8 MHz) of the C-1 region of 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose (**1**) prepared (a) from D-[U- $^{13}\text{C}$ ]glucose, (b) after biosynthesis with D-[U- $^{13}\text{C}$ ]glucose, and (c) after biosynthesis with D-glucose + D-[U- $^{13}\text{C}$ ]glucose.

**Table 1.** Interpretation of Experimental Data. All probabilities for the Two-Site Groups. Resulting Correlation Terms with the Three-Site Cases and Probabilities with the Four-Site Group (1-4)

<i>P</i> values	$P_{12} = 0.89$ , $P_{23} = 0.60$ , $P_{34} = 0.26$ , $P_{45} = 0.95$ , $P_{56} = 0.93$
<i>C</i> values	$C_{13} = 0.066$ , $C_{24} = 0.104$ , $C_{35} = 0$ , $C_{46} = 0$
probabilities for sites 1-4	$P_{1234} = 0.26$ , $P_{123/4} = 0.34$ , $P_{12/3/4} = 0.29$ , $P_{1/2/3/4} = 0.11$

transformation into 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose<sup>10</sup> (**1**). This compound was chosen since the chemical shifts between C-2, C-3, C-4, and C-5 are large enough to suppress most of the second-order effects in the  $^{13}\text{C}$  spectrum.<sup>4</sup> Figure 1 gives the experimental spectra of C-1 for this compound obtained from the D-[U- $^{13}\text{C}$ ]glucose starting material and from two biosynthetic experiments using this precursor. One experiment was performed with D-[U- $^{13}\text{C}$ ]glucose as precursor; the result has been analyzed with  $\tau = 0.72$  ( $\rho_A = 0.87$ ,  $\rho_B = 0.01$ ,  $\alpha = 0.83$ ); the value of  $\rho_B$  takes into account every additional source of carbon atoms. The other experiment was carried out with a 1:1 mixture of D-[U- $^{13}\text{C}$ ]glucose and unlabeled glucose; the result was analyzed with  $\tau = 0.35$  ( $\rho_A = 0.87$ ,  $\rho_B = 0.01$ ,  $\alpha = 0.40$ ). All of the values of *P* and *C* given in Table I come from this last experiment for which 200 mg of labeled precursor was used. Figure 1 shows clearly that the experimental determination of the percentages of isotopomers is easy and that these percentages are very different in the three given spectra. The contributions of all of the isotopic species can be expressed mathematically, using a Fortran program MARQUAG,<sup>11</sup> by means of terms such as  $\rho_A$ ,  $\rho_B$ ,  $\alpha$ , and the probabilities defined precendently. Another especially written program (LAOCISOM)<sup>12</sup> must be used in order to check this by reconstruction of the NMR spectrum from each carbon site.

All the results are summarized in Table I, where it can be seen that, for the group of four atoms C-1-C-4, the maximum values chosen for  $C_{13}$  and  $C_{24}$  give only four nonzero proba-

bilities. The values of  $P_{45}$  and  $P_{56}$  very close to 1 do not allow us to determine experimentally the terms  $C_{35}$  and  $C_{46}$  and, so, the remaining probabilities have been estimated statistically (using a value of 0 for these two correlation terms). From these values the percentages of isotopomers in both biosynthetic experiments were calculated and the agreement with independent mass spectra is good.<sup>4</sup>

This particular treatment seems very promising for the interpretation of biosynthetic mechanisms, since the probability of occurrence of any sequence of the atoms in the skeletal molecule can be determined, allowing us to predict the results for a precursor selectively enriched at a particular position.<sup>4</sup>

It must be pointed out that this approach provides experimental data only on the probabilities for groups of two, three, or more atoms which come from the same precursor molecule, but nothing on the location of these atoms within this molecule. However it is a very general tool; the only limitation of the proposed mathematical treatment is that the percentage of labeling for these groups does not depend on the carbon atoms considered—that is to say that a statistically enriched precursor (used alone or, better, in a mixture with an equivalent amount of unlabeled compound) gives a statistically enriched biosynthesized compound.

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