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Camptothecin derivatives are clinically used antitumor alkaloids that belong to monoterpenoid indole alkaloids. In this study, we investigated the biosynthetic pathway of camptothecin from [1-13C]glucose (Glc) by in silico and in vivo studies. The in silico study measured the incorporation of Glc into alkaloids using the Atomic Reconstruction of Metabolism software and predicted the labeling patterns of successive metabolites from [1-13C]Glc. The in vivo study followed incorporation of [1-13C]Glc into camptothecin with hairy roots of Ophiorrhiza pumila by 13C nuclear magnetic resonance spectroscopy. The 13C-labeling pattern of camptothecin isolated from the hairy roots clearly showed that the monoterpeno-secolloganin moiety was synthesized via the 2C-methyl-erythritol 4-phosphate pathway, not via the mevalonate pathway. This conclusion was supported by differential inhibition of camptothecin accumulation by the pathway-specific inhibitors (fomidomycin and lovastatin). The quinoline moiety from tryptophan was also labeled as predicted by the Atomic Reconstruction of Metabolism program via the shikimate pathway. These results indicate that camptothecin is formed by the combination of the 2C-methyl-erythritol 4-phosphate pathway and the shikimate pathway. This study provides the innovative example for how a computer-aided comprehensive metabolic analysis will refine the experimental design to obtain more precise biological information.

Camptothecin, a plant-originated alkaloid, exhibits an antitumor action due to its inhibitory activity to DNA topoisomerase I (Hsiang et al., 1985). At present, semisynthetic water-soluble camptothecin analogues, topotecan and irinotecan, are prescribed as clinical antitumor agents throughout the world. Despite its quinoline structure, camptothecin belongs biogenetically to a family of modified monoterpenoid indole alkaloids. All monoterpenoid indole alkaloids are synthesized from strictosidine, a universal biosynthetic intermediate that is formed by condensation of tryptamine with secolloganin, a monoterpeno-glucoside (Stöckigt and Zenk, 1977; Stöckigt and Ruppert, 1999). Thus, secolloganin is a key metabolite, which occupies a pivotal position in several biosynthetic pathways and acts as a central compound for the formation of a variety of natural products (Kutchan, 1993). In the late 1970s, biosynthetic studies of camptothecin using 14C and 3H tracers with Camptotheca acuminata revealed strictosamide, a product of intramolecular cyclization of strictosidine, as a penultimate precursor (Hutchinson et al., 1979). Since that study, however, no substantial progress has been made on biosynthetic mechanism of camptothecin, presumably because of the lack of a suitable experimental system for biosynthetic research. Hairy roots of Ophiorrhiza pumila (Rubiaceae) produce a high level of camptothecin and excrete it into medium in a large quantity (Saito et al., 2001; Kitajima et al., 2002; Sudo et al., 2002). This hairy root culture is a desirable experimental system for research of molecular biology and biochemistry of camptothecin biosynthesis (Yamazaki et al., 2003a, 2003b).

For the formation of isopentenyl diphosphate (IPP), the precursor of terpenoid biosynthesis, the mevalonate (MVA) pathway has been known since the 1950s (for review, see Cane, 1999). Recently, the 2C-methyl-d-erythritol 4-phosphate (MEP) pathway, in which IPP is formed from 1-deoxy-d-xylulose 5-phosphate by condensation of glyceraldehyde 3-phosphate and pyruvate, was found to be present in many eubacteria, green algae, and plastids of
plants (Fig. 1; for review, see Rohmer, 1999; Rodriguez-Concepción and Boronat, 2002; Kuzuyama and Seto, 2003). In plants, the formation of IPP and its isomer, DMAPP, is compartmentalized: Although the MVA pathway is localized in the cytosol, the MEP pathway operates in plastids (Eisentreich et al., 2001; Rodriguez-Concepción and Boronat, 2002). Lovastatin, a highly specific inhibitor of hydroxymethylglutaryl (HMG)-CoA reductase in the MVA pathway, strongly inhibits sterol biosynthesis in higher plants (Alberts et al., 1980; Fig. 1). Fosmidomycin, a specific inhibitor of 1-deoxy-d-xylulose 5-phosphate reductoisomerase in the MEP pathway, blocks the MEP pathway (Kuzuyama et al., 1998). These pathway-specific inhibitors can be used to perturb biosynthetic flux in hairy roots of *O. pumila*.

To analyze the metabolic network involved in camptothecin biosynthesis, the Atomic Reconstruction of Metabolism (ARM; Arita, 2003) software has been adopted. In the ARM software, compound structures are represented as graphs to detect their structural changes in catalyzed reactions. These changes are semiautomatically detected by graph comparison, and each reaction is analyzed at the atomic scale. The software consists of three modules: the database for atomic correspondents in enzymatic reactions, the tracing engine, and the graphical user interface. Atomic traces of carbon and nitrogen in the input reactions are analyzed by a structure-matching algorithm in advance and are precompiled in the database as a set of atomic position pairs between metabolites. Similarly, reactants are analyzed for their structural symmetry in advance. By combining information on structural correspondence and symmetry thus computed, the tracing engine can track any carbon or nitrogen atom in the metabolic network represented as a set of precompiled reactions. The tracing is performed qualitatively by a shortest paths algorithm that can output all possibilities (not only the shortest one) through the network. The software is used as a verification tool of the reconstructed metabolic network against observed data in isotope tracer experiments: The software rationalizes isotope-labeled positions in the metabolic network. This is the only software available offering automated tracer experiments in silico. Actual application of the ARM software to predict the labeling pattern of in vivo tracer experiments is an intriguing issue as a merger of bioinformatics and experimental biochemistry in the newly developing metabolomics research.

This paper describes the in silico and in vivo studies of the biosynthesis of camptothecin with the hairy roots of *O. pumila*. The tracer experiments using [1-13C]Glc in silico and in vivo and perturbation of

![Figure 1](https://example.com/figure1.png)
the pathways by specific inhibitors indicate that the secologanin moiety is derived from the MEP pathway. The metabolic flux from [1-13C]Glc to camptothecin via central sugar metabolism, amino acid biosynthesis, and the MEP pathway is also discussed by combining the in silico and in vivo studies. This study is one of the first successful examples, to our knowledge, of integration of computational metabolomics and experimental biochemistry.

RESULTS

In Silico Analysis

The atomic traces were computed by the ARM software. Although metabolic data of over 2,000 reactions are compiled in the database, because sufficient sugar was supplied in our in vivo experimental conditions, biochemical pathways of amino acids and other metabolites that do not directly contribute to the secologanin biosynthesis are excluded from the current analysis. The ARM program actually computed a reconstructed network of 131 reactions and 280 mappings. Because the network contains many loops, the analysis here is described for each functional block as depicted in Figure 2. The software and data are available at http://www.metabolome.jp/.

Glycolysis, Pentose-Phosphate, and Some Amino Acid Pathways

Breakdown of [1-13C]Glc by glycolysis provides [2-13C]acetyl-CoA, the direct precursor of the MVA pathway, and [3-13C]glyceraldehyde 3-phosphate and [3-13C]pyruvate, two precursors of the MEP pathway (Fig. 3). Because the reactions of glycolysis and triose phosphate isomerase are reversible and interconvert dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, both C-1 and C-6 positions of Glc 6-phosphate are expected to be labeled as shown in Figure 3. The linear portion of the pentose phosphate pathway produces [5,13C]ribulose 5-phosphate, at first, by oxidation and decarboxylation of [1,6-13C]Glc 6-phosphate. By the pentose phosphate pathway, the C-1 of ribulose 5-phosphate is also 13C-labeled in addition to C-5 because the C-1 is subsequently derived from the C-1 of xylulose 5-phosphate, the C-1 of sedoheptulose 7-phosphate, and the C-1 of Fructose 6-phosphate. The remainder of the pentose phosphate pathway accomplishes conversion of the [1,5-13C]ribulose 5-phosphate to the [1,5-13C]Rib 5-phosphate.

The C-1 and C-6 positions of Glc both reach the C-3 position (the enol terminal) of pyruvate and further diffuse to beta carbons in amino acids including Ala, Ser, Asp, and so on. By an exhaustive computation with the ARM program, these labeled positions are shown to be invariable throughout the defined metabolism.

Tricarboxylic Acid (TCA) Cycle

After the application of pyruvate decarboxylase, the acetyl moiety of pyruvate enters the TCA cycle. The two carbons of the acetyl moiety are incorporated during the first cycle, and they are lost as carbon dioxide in successive cycles. In principle, the C-3 position of pyruvate is identical to either C-2 or C-3 position of oxaloacetate by the label-scrambling effect at succinate. If the C-2/C-3-labeled oxaloacetate is taken back to the gluconeogenesis, C-1, C-2, C-5, and C-6 of Glc would be labeled. However, presumably due to a limited extent of gluconeogenesis occurring under our experimental conditions, the
labeling of Glc only occurred at C-1 and C-6 positions (see later).

Trp Biosynthesis

Trp is synthesized through shikimate, whose carbon backbone consists of erythrose 4-phosphate and phosphoenolpyruvate (Fig. 4). Shikimate is then transformed to anthranilate and eventually to indole by integrating C-1 and C-2 of Rib 5-phosphate. Thus, three carbons should be labeled in the indole ring from [1-\(^{13}\)C] Glc. These carbons are derived from C-4 of erythrose 4-phosphate, C-3 of phosphoenolpyruvate, and C-1 of Rib 5-phosphate. The final step in the Trp biosynthesis is the condensation of indole with a Ser moiety. Ser is synthesized through glycerate, and its beta carbon labeled with \(^{13}\)C is derived from C-1 or C-6 of Glc as mentioned.

Secologanin and Camptothecin Biosynthesis

Through the MVA pathway, IPP and DMAPP are distinctly labeled at C-2, C-4, and C-5 from [1-\(^{13}\)C]Glc as indicated in Figure 1A. By the MEP pathway, in contrast, IPP and DMAPP are labeled at C-1 and C-5 as shown in Figure 1B. Secologanin is produced from IPP and DMAPP successively via geranyl diphosphate, 10-hydroxygeraniol, and loganin (Fig. 5). The randomization of two carbons derived from C-4 and C-5 of DMAPP may be taken into account during the conversion of 10-hydroxygeraniol to loganin (Coscia et al., 1970; Contin et al., 1998; Eichinger et al., 1999). The condensation of secologanin and tryptamine yields strictosidine. The labeled positions of camptothecin from [1-\(^{13}\)C]Glc can be predicted either by the MVA pathway or the MEP pathway as shown in Figure 5.

In Vivo Study

Culture Conditions

To achieve sufficient incorporation of Glc and growth of cells, the sugar composition in the Gamborg B5 culture medium was optimized: the combinations of 2% (w/v) Suc, 1% (w/v) Suc plus 1% (w/v) Glc, 2% (w/v) Glc, 3% (w/v) Glc, and 4% (w/v) Glc were tested with the hairy roots of O.
pumila. The medium with 2% (w/v) Suc gave the highest production of camptothecin. In the medium containing 1% (w/v) Suc plus 1% (w/v) Glc, the growth rate decreased compared with the condition of 2% (w/v) Suc (data not shown). However, the content of camptothecin per fresh weight of tissues was the same as that in the medium with 2% (w/v) Suc. In the medium containing only Glc, both growth rate and camptothecin content decreased. Thus, the medium composition of Gamborg B5 salt and vita-

mins with 1% (w/v) Glc plus 1% (w/v) Suc was chosen for feeding experiments with [1-13C]Glc.

Incorporation of [1-13C]Glc into Camptothecin

The hairy roots of *O. pumila* were grown in the presence of [1-13C]Glc for 4 weeks. Camptothecin was isolated from the hairy roots and the culture medium by successive separation by chromatography. Purified camptothecin was analyzed by 1H- and 13C-NMR spectroscopy. All 13C signals could be assigned by the technique of two-dimensional NMR spectroscopy. Figure 6 shows the 13C spectra of the standard camptothecin and the 13C-enriched compound determined in dimethyl sulfoxide (DMSO). The relative 13C intensities and enrichments for all carbons of camptothecin in DMSO-d6 and CDCl3-CD3OD are presented in Table I.

In the experiments with [1-13C]Glc, the signals corresponding to C-2, C-6, C-9, C-13, C-14, C-16a, C-17, C-18, and C-21 of camptothecin exhibited clear 13C enrichment (Fig. 6; Table I), whereas the other carbons were virtually not labeled. The enrichment of C-2, C-16a, and C-17 was apparently less than the rest of labeled carbons. In the 1H-NMR spectrum, satellite signals due to incorporation of 13C appeared at H-9, H-14, H-17, and H-18 of camptothecin (data not shown), supporting the incorporation pattern deduced from the 13C spectra. All spectral data demonstrated that, in camptothecin biosynthesis from [1-13C]Glc, the Trp-derived quinoline moiety was labeled as predicted by the ARM analysis and the secologanin-derived part was labeled according to the predicted pattern via the MEP pathway. No sign of the operation of the MVA pathway was indicated.

The absolute 13C abundances calculated from the intensities of these 13C-coupled 1H-satellite signals were following: 14.3% for H-18 derived from C-5 of IPP, 14.6% for H-14 derived from C-5 of DMAPP, 7.2% for H-17 derived from randomized C-4 and C-5 of DMAPP (see later), and 13.3% for H-9 derived from C-4 of tryptamine; these corresponded to 13C excess to natural abundance of 13.2%, 13.5%, 6.1%, and 12.2%, respectively. These results indicated a specific incorporation rate of 12% to 14% from [1-13C]Glc into appropriate positions and randomization of C-17 and C-16a reaching almost to equilibrium.

Effect of the Pathway-Specific Inhibitors

Lovastatin and fosmidomycin were added to the 3-week-old hairy roots. Three days after the addition of the compounds, growth rate and camptothecin content were measured (Fig. 7). Treatment with 100 μM fosmidomycin, a specific inhibitor of the MEP pathway, resulted in significant decrease of camptothecin production (*P* < 0.01), although growth rate was slightly improved. In contrast, lovastatin, a specific inhibitor of the MVA pathway, did not affect the growth and even exhibited slightly a trend of stimulation of the camptothecin production. These results further support the conclusion that the secologanin moiety of camptothecin is derived from the MEP pathway but not the MVA pathway.

**DISCUSSION**

In the present study, the biosynthetic pathway of camptothecin from [1-13C]Glc was investigated by in silico computation using the ARM program and by in vivo tracer experiments. Combining all results obtained together, we conclude that secologanin moiety is derived by the MEP pathway and that Trp part is formed by the general shikimate pathway.

It is now generally accepted that the MEP pathway is responsible for the formation of IPP and DMAPP in plastids, whereas the MVA pathway produces cytosolic IPP (Eisenreich et al., 2001; Rodriguez-Concepción and Boronat, 2002). Most cyclic monoterpenes are produced via the MEP pathway because geranyl diphosphate synthase and monoterpen cyclase are localized in plastids (Wise and Croteau, 1999). However, the monoterpen moiety in shikonin is exceptionally derived via the MVA route (Li et al., 1998). The present study clearly indicated that monoterpenoid secologanin moiety of camptothecin is formed by the MEP route. This is in good agreement with the previous findings that both secologanin (Contin et al., 1998) and loganin (Eichinger et al., 1999) are formed by the MEP pathway in *Catharanthus roseus* and *Rauwolfia serpentina*, respectively, which also produce the monoterpenoid indole alkaloids.

From the final incorporation pattern of 13C into camptothecin, the isotopic randomization of the carbons derived from C-5 (Z) and C-4 (E) methyl groups of DMAPP was observed. The C-16a derived from C-5 of DMAPP and C-17 from C-4 of DMAPP in camptothecin were both labeled to nearly equal extents by approximately 50% of the rest of labeled carbons from secologanin deduced from intensities of 13C signals and 13C-coupled 1H-satellite signals. These indicated that C-5 of DMAPP initially labeled with 13C was randomized with C-4 in the course of camptothecin formation. The same randomization was also observed in the biosynthesis of secologanin in *C. roseus* (Contin et al., 1998) and loganin in *R. serpentina* (Eichinger et al., 1999), suggesting that it should take place before loganin formation. Because loganin biosynthesis is postulated to proceed via iridodial intermediate as shown in Figure 5 (Usasato et al., 1986), randomization of methyl groups occurs most likely at this intermediate. The randomization of smaller extent (<10%) respecting methyl group of 2C-methyl-p-erythritol 2,4-cyclodiphosphate in the course of formation of IPP and DMAPP was also reported (Fellmermeier et al., 2001). This randomiza-
Reaction was observed not only with DMAPP but IPP as well regarding C-5 and C-4. Because IspG and IspH proteins are involved in the formation of IPP and DMAPP (Rohdich et al., 2003), the randomization takes place either during these step-wise reactions or IPP/DMAPP isomerase reaction. However, with re-

**Figure 6.** 125 MHz $^{13}$C-NMR spectra of camptothecin in dimethyl sulfoxide (DMSO). Numbers indicate the assignments of carbon positions of camptothecin. Asterisks, $^{13}$C-enriched signals. A, Standard camptothecin. B, $^{13}$C-enriched camptothecin be feeding with [1-$^{13}$C]Glc.
spect to the camptothecin carbons, C-18 derived from C-5 of IPP and C-3 from C-4 of IPP, C-18 was exclusively labeled by [1-13C]Glc but no 13C-enrichment at C-3. These suggested that no apparent randomization took place in the formation of IPP.

It is generally expected that the C-1 position of Glc is interconvertible with C-6 of Glc in the central sugar metabolism because the reactions from Glc 6-phosphate to glyceraldehyde 3-phosphate and dihydroxyacetonephosphate are all reversible (Buchanan et al., 2000). In addition, the reactions involved in the pentose phosphate pathway also are presumed to be equilibrated (Buchanan et al., 2000). These assumptions were validated by the incorporation pattern of 13C into camptothecin. The labeling of secologanin moiety fed by [1-13C]Glc indicated the incorporation of 13C into pyruvate and glyceraldehyde 3-phosphate due to interconversion between glyceraldehyde 3-phosphate and dihydroxyacetonephosphate by triosephosphate isomerase. The equilibrium of triose phosphate/pentose phosphate pool was confirmed by equal 13C incorporation into the Trp moiety compared with the secologanin moiety. The Trp moiety is derived from the metabolites of glycolysis and of the pentose phosphate pathway (Fig. 4). However, the lower enrichment of C-2 of camptothecin may suggest that pentose phosphate pathway was not fully equilibrated. The full extent of labeling at C-1 of Rib 5-phosphate leading of C-2 of camptothecin can be achieved only by full equilibrium of pentose phosphate pathway.

If the C-2/C-3-labeled oxaloacetate derived from the TCA cycle is taken in the gluconeogenesis, the [1,2,5,6-13C]Glc would be generated. However, this distribution was not observed; isotope labels were found only at the positions derived from C-1 and C-6 of Glc, indicating a negligible back flux from the TCA intermediates to gluconeogenesis. This observation would be reasonable because sufficient sugar was supplied in the present experimental conditions.

Investigation on metabolic network using [U-13C6]Glc into amino acids, sterols, and starch was carried out recently with maize (Zea mays) kernels using 13C-NMR (Glawischnig et al., 2001, 2002). Relative contributions of separate pathways for the formation of specific metabolites (i.e. Ser, Lys, and sitosterol) could be determined. They observed the extensive recycling of Glc before its incorporation into starch via glycolytic, glucogenic, and pentose phosphate pathways. These experimental NMR tracer studies can be integrated with a computation as shown in the present study for verification of a given hypothesis by more logical ways.

The ARM software allows us to search for pathways containing as many reactions as possible and to trace specific atoms (carbon or nitrogen) because the mapping information is on the atomic scale. The analysis can be interactively verified on the Web site. Although the reactions computed in the present study were 131 reactions, data for more than 2,000 reactions are available on the Web (http://www.metabolome.jp), and labeling analysis using 2,000

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a Signal positions determined in DMSO-d6. b Relative intensities of signals to C-7 set as 1 determined in DMSO-d6. c Recorded in DMSO-d6 or CDCl3-CD3OD. d n.d., Not determined due to signal overlapping in CDCl3-CD3OD.

Camptothecin Biosynthesis in Silico and in Vivo

Plant Cell Culture and Feeding Experiments

The hairy roots of Ophiopogon pumila accumulating camptothecin (Saito et al., 2001) were maintained in Gamborg B5 salt medium (Gamborg et al., 1968) supplemented with B5 vitamins and 2% (w/v) Suc. They were subcultured every 3 weeks by inoculation of 400 to 600 mg of hairy root in 100-mL Erlenmeyer flasks containing 50 mL of medium at 25°C on a rotary shaker (60 rpm) under dark condition. For the optimization of culture condition in the presence of Glc, 400 to 600 mg of hairy root per flask was subcultured on 50 mL of the same medium, containing 1% (w/v) Suc plus 1% (w/v) Glc, 2% (w/v) Glc, 3% (w/v) Glc, or 4% (w/v) Glc. For the feeding experiments, the hairy roots were subcultured on 50 mL of the same medium, 20 flasks, containing 1% (w/v) Suc plus 1% (w/v) 1-[1-13C]Glc (95% enriched, Cambridge Isotope Lab., Andover, MA). After 4 weeks, the hairy roots were harvested for extraction of camptothecin.

Isolation of Camptothecin

The hairy roots fed with [1-13C]Glc were extracted with hot methanol. The resulting extract was evaporated to dryness, and the residue was dissolved in water and extracted with chloroform. The culture medium of hairy roots was also extracted with chloroform. The combined organic phase was then evaporated to dryness, and the residue was dissolved in a mixture of chloroform:methanol:water (10:5:1 [v/v]). The material was subjected to separation by HPLC using an AQUASIL SS-752N column (10 × 250 mm, Senshu Scientific, Tokyo) with a solvent system of chloroform:methanol:water (10:5:1 [v/v]). The camptothecin fraction was subjected to further separation by medium pressure liquid chromatography using a silica gel CPS-HS-221-5 column (22 × 100 mm, Kusano Kagakukikai, Tokyo) with a solvent system of chloroform:methanol (95:5 [v/v]). Final purification of camptothecin was performed by HPLC using an AQUASIL SS-752N column with a solvent system of chloroform:methanol (98:2 [v/v]). The yield of pure camptothecin was approximately 1 mg from 20 flasks containing 50 mL of culture medium in each flask.

NMR Spectroscopy

1H- and 13C-NMR spectra of camptothecin were recorded in DMSO-d6 and CDCl3:CD3OD (5:1 [v/v]) at 500 and 125 MHz, respectively, using a JEOL JNM A-500 spectrometer (Tokyo). The measurement conditions for 1H were as follows: 45° pulse, 6 µs; repetition time, 3 s; spectral width, 10 kHz; temperature, 28°C; and broadness factor, 0.01 Hz. The measurement conditions for 13C were as follows: 45° pulse, 4 µs; repetition time, 1.5 s; spectral width, 30 kHz; temperature, 28°C; and broadness factor, 2.00 Hz. The assignment of 13C-NMR chemical shifts for camptothecin was carried out by two-dimensional experiments with literature data (Lin and Cordell, 1990; Ezell and Smith, 1991). Relative 13C abundance of individual carbon atoms was calculated by comparison of 13C signal integrals between 13C-labeled and unlabeled camptothecin.

Inhibitor Experiments

The stock solution of lovastatin (Wako, Osaka) was prepared as described previously (Han et al., 2002). Fosmidomycin was kindly gift from Dr. Tomohisa Kuzuyama (University of Tokyo) and used as a 10 or 1 mM stock solution in 50% (v/v) ethanol. These stock solutions were filter sterilized and added to the hairy roots cultures containing 50 mL of medium at d 21 after inoculation. For the control, 0.5 mL of 50% (v/v) ethanol was used. After 3 d, hairy roots were harvested and camptothecin content was determined as described previously (Saito et al., 2001).

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Figure 7. Effect of pathway inhibitors on camptothecin accumulation. The inhibitors, lovastatin and fosmidomycin, were added to the 3-week-old hairy roots at the final concentrations indicated. Three days after the addition of the compounds, the hairy roots were extracted for determination of camptothecin. Bar = SD of triplicate determinations. The difference of camptothecin production among control and treated samples was statistically significant by Student’s t test (**, P < 0.01).

MATERIALS AND METHODS

In Silico Analysis

From a set of reaction formulas and metabolite structures involved, the ARM software (Arita, 2003) can reproduce the tracing of carbon and nitrogen atoms in the reconstructed network. To analyze the camptothecin biosynthesis from Glc, we selected 131 reactions covering the glycolysis, pentose-phosphate pathway, TCA cycle, biosynthesis/degradation of amino acids (Gln, Glu, Trp, Ser, Ala, Asp, Thr, Cys, and Met), and secologanin biosynthesis through both MVA and MEP pathways. Also integrated were some spontaneous or hypothetical reactions whose enzymes are yet undefined. Alternative pathways were also investigated using all reactions (over 2,000) in the database. Reversibility of the reactions was confirmed to the arrow directions in the Biochemical Pathway Chart (Michal, 1999). Computed atomic traces in reactions were manually verified for their biochemical correctness. By assembling the computed traces with the reversibility information, the software can reproduce all reachable atomic positions that originated in the isotope-labeled atoms, i.e. C-1 position of Glc in the NMR analysis. The used dataset and the pathway-computation software are accessible from the Web site at http://www.metabolome.jp/.
LITERATURE CITED


