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# PRECURSORS OF THE $C_{9-10}$ UNIT OF CEPHAELINE

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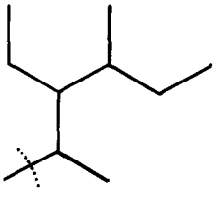
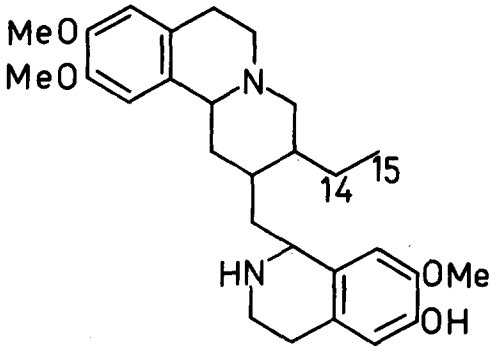
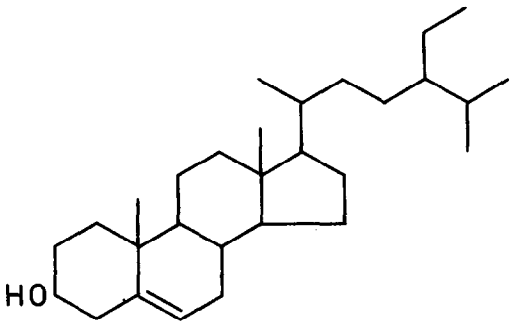
Although the $C_{9-10}$ unit (I), which occurs in several classes of alkaloids, is biosynthetically derived from geraniol (1,2,3,4), and from mevalonic acid (5), acetic acid is a poor and non specific precursor of this unit (6,7,8). Recently we reported the novel and specific incorporation of glycine into the $C_{9-10}$ unit in cephaeline (II), but found that glycolic acid was no more specific as a precursor than was acetic acid (9). We now present further evidence that glycine is specifically incorporated into the $C_{9-10}$ portion of cephaeline, but is not incorporated into β-sitosterol (III), produced in the plant at the same time. Alternatively, acetic acid acts as a specific precursor of β-sitosterol in the normal manner, but is neither significantly nor specifically incorporated into cephaeline.



I




II




III

Radioactive compounds were fed by the wick method to four three-year old Cephaelis acuminata plants, and the plants were harvested either after 10 or after 21 days. Cephaeline and β-sitosterol were isolated from the roots of each group of plants, and examined for radioactivity.

When glycine-2-$C^{14}$ (125 μc) was fed for 10 days cephaeline (20 mg) with a specific activity of 1.1 x $10^5$ d p m/mM (0.005% incorporation) was obtained. It was subjected to a Kuhn-Roth oxidation to yield sodium acetate ($C_{14}$ and $C_{15}$) containing 18% of the activity of the cephaeline. A Schmidt reaction on the sodium acetate yielded methylamine, (isolated as the N-methylbenzamide), which contained all the activity of the sodium acetate. The carbon dioxide was inactive. These results are in agreement with earlier findings (9). The activity of the cephaeline (12 mg) derived when glycine-2-$C^{14}$ (125 μc) was fed for 21 days was considerably lower (8.7 x $10^3$ d p m/mM) than that from the 10 day feeding. This may well indicate a significant turnover of cephaeline in the plants.

When sodium acetate-2-$C^{14}$ (125 μc) was fed, either for 10 or for 21 days, the cephaeline obtained (7 and 12 mg respectively) was of very low activity (1.7 x $10^3$ d p m/mM in each experiment). A larger feeding of precursor (250 μc) for 10 days produced sufficient cephaeline for degradation purposes (60 mg, 7.7 x $10^3$ d p m/mM). It was subjected to the Kuhn-Roth oxidation to give sodium acetate (1.0 x $10^3$ d p m/mM) containing 13.5% of the activity of the cephaeline. In this case, however, the activity was equally distributed between $C_{14}$ and $C_{15}$ (methylamine 0.5 x $10^3$ d p m/mM, barium carbonate 0.5 x $10^3$ d p m/mM).

The sterol fraction was separated by extraction from each of the groups of plants fed, and in each case β-sitosterol was isolated by scavenging with inactive β-sitosterol (5 mg). When glycine-2-$C^{14}$ (125 μc) was fed for 10 days the resulting β-sitosterol was essentially inactive ($2.5 \times 10^3$ d p m/mM), and it was still of low activity when a similar feeding was allowed to continue for 21 days ($8.7 \times 10^3$ d p m/mM). However, when sodium acetate-2-$C^{14}$ was fed for 10 or 21 days, the β-sitosterol was of considerably higher activity ($1.8 \times 10^4$ and $9.2 \times 10^4$ d p m/mM respectively). The β-sitosterol isolated from the 21 day feeding of sodium acetate-2-$C^{14}$ was subjected to a Kuhn-Roth oxidation to yield sodium acetate which contained 7.2% of the activity of the β-sitosterol on a mole per mole basis. This agrees well with the value of 6.6% expected if this sterol is biosynthesized in the normal manner.

These results indicate that sodium acetate is converted into β-sitosterol in Cephaelis acuminata in a manner consistent with that normally found for steroid biosynthesis. Glycine, as might be expected, cannot efficiently replace acetic acid as a source of the two carbon unit for this purpose. The $C_{9-10}$ unit is now generally accepted to be of monoterpene origin, and has been shown to be biosynthetically derived from geraniol in cephaeline (10). In spite of this, radioactive acetic acid cannot label this unit with any specificity or efficiency, such activity as it contributes being well randomized. Glycine-2-$C^{14}$ can, however, act as an efficient, and apparently a specific, two carbon precursor of the $C_{9-10}$ unit of cephaeline, delivering 15-18% of the activity incorporated to $C_{15}$ but none to $C_{14}$. This is the first direct evidence for this sort of a distinction between glycine and acetic acid, wherein they act as specific and exclusive precursors of different monoterpene moieties, in different compounds, in the same plant. It strongly supports the idea that the oxidation state of a two carbon compound may exert a major effect on determining its utilization in biosynthesis.

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