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BIOSYNTHESIS OF CAMPTOTHECIN. II. CONFIRMATION THAT ISOVINCOSIDE, NOT VINCOSIDE, IS THE PENULTIMATE BIOSYNTHETIC PRECURSOR OF INDOLE ALKALOIDS

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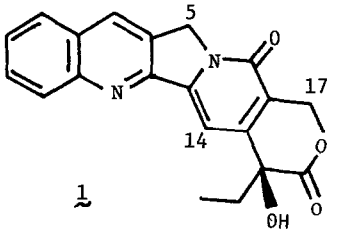
We have been able to define partially the biosynthetic pathway of camptothecin (1) based on the results of feeding experiments with ^{13}C, ^{14}C, and ^{3}H labeled precursors.[1] Thus, it appears that 1 is formed *in vivo* from tryptophan and mevalonic acid *via* isovincoside lactam (strictosamide), 3a, and/or its 18,19-dihydroanalog, 3b. Vincoside lactam (3d), on the other hand, is not significantly incorporated into 1. These results are at variance with the data of Battersby *et al.*[2,3] who had shown several years earlier that vincoside (2c) was the penultimate biosynthetic precursor of two major classes of indole alkaloids found in other higher plants. At the time we obtained our earlier data and the newer information reported here[4], it did not seem surprising that the C(3)S isomer of 2 and 3 was the precursor of 1 in a plant botanically quite unrelated to those used in Battersby's investigations. However in view of the recent startling results from M. H. Zenk's group at Bochum[5], wherein isovincoside (2a), *not* vincoside, has been demonstrated to be specifically incorporated into several classes of indole alkaloids in whole plants and in cell-free systems derived therefrom - notably *Catharanthus roseus*, it is important to officially describe the experimental confirmation of our earlier presumption[1] that 2a is the precursor of 1, as well as some additional details concerning the biosynthesis of 1.



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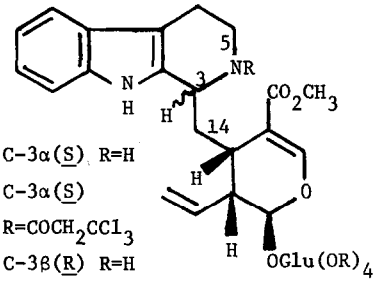
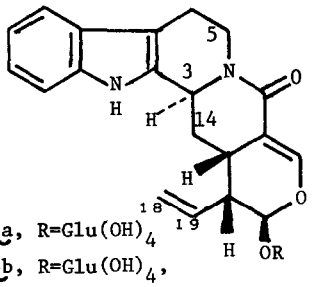


2a, C-3α(S) R=H
2b, C-3α(S) R=$COCH_2CCl_3$
2c, C-3β(R) R=H
2d, C-3β(R) R=$COCH_2CCl_3$



3a, R=$Glu(OH)_4$
3b, R=$Glu(OH)_4$, 18,19-H_2
3c, R=H
3d, C(3)β, R=$Glu(OH)_4$

Radiochemical precursor synthesis was done as follows. [14-$^{3}H_2$]-2 was synthesized by condensation of secologanin[3] and tryptamine hydrochloride in 0.2 M phosphate buffer, pH ca. 5, containing T_2O (200 mCi), at 37°C for 72 hr. From the resulting C(3) epimeric mixture [14-$^{3}H_2$]-2a and [14-$^{3}H_2$]-2c were obtained *via* 2b and 2d using the methodology of Mattes *et al.*[3] The regiospecificity of ^{3}H labeling was proven using D_2O; deuteration was seen to have occurred only at C(14) by ^{1}H and ^{13}C NMR analysis of tetraacetyl 3a and 3d. Samples of [14-$^{3}H_2$]-3a and 3c were prepared

from [14-$^3H_2$]-2a using literature methods[2,7] by which the regiospecific ^3H labeling was not altered. Precursor radiochemical purity was assessed by crystallization when possible, or by repeated TLC to constant specific radioactivity.

Parallel feeding experiments were done with apical cuttings of Camptotheca acuminata in July and August of 1975. The essential experimental data and results are shown in the following Table.

Precursor	Radioactivity Administered[a] (dpm)	Radioactivity of Isolated 1 (dpm)	Absolute[b] (Specific)[c] Incorporation (%)
[14-$^3H_2$,5-^{14}C]-3a[d]	3.3×10^7 (^{14}C) [$^3H/^{14}C$=2.55]	3.76×10^5	1.12 (0.52) [$^3H/^{14}C$=2.31]
[14-$^3H_2$]-2a	2.01×10^8	2.4×10^5	0.12 (0.051)
[14-$^3H_2$]-2c[e]	2.26×10^8	2.94×10^4	0.013 (0.0074)
[14-$^3H_2$]-3c	1.65×10^9	3.8×10^2	0.00002 (0.00001)

(a) Precursors were administered in aqueous solution or suspension (Tween 20) to the cuttings, which then were maintained in a humid growth chamber at 20°C under 300-400 μeinsteins M^{-2} sec^{-1} illumination for 10-14 days. The isolation of 1 was done by standard methods and it was recrystallized from $CHCl_3$-MeOH-EtOAc (8:2:1) to constant specific radioactivity. In all cases >90% of the administered radioactivity was absorbed by the cuttings. (b) Total dpm of 1 isolated divided by total dpm of precursor fed x 100. (c) Dpm/mmol 1 isolated divided by dpm/mmol precursor fed x 100. (d) Synthesized from [1-^{14}C] tryptamine via [5-^{14}C]-3a by admixture.[1] (e) Some [14-$^3H_2$]-2a may have been present.

The apparent incorporation of 2c into 1 is presumed to be due to a small contamination with 2a[8], but it is clear that isovincoside (2a) is a much better precursor of 1 than vincoside (2b), consistent with the utilization of 3a in the subsequent biosynthetic transformations. Since isovincoside aglucone (3c)[7] was not significantly incorporated into 1, the biosynthetic sequence suggested earlier[1] must not involve the conversion of 3a to 3c before formation of the pyrrolo [3,4-b] quinoline ring system. Finally, since chemical degradation of doubly-labeled 1 (from 3a) showed that ^3H was not located at C(17)[4], the reason for the curious lack of significant ^3H loss from C(14) of 3a in vivo remains to be established.

Future experimental work and written discussions on indole alkaloid biosynthesis will have to accede the no longer enigmatic stereochemical relationship at C(3) of the biogenetically penultimate glycoalkaloids, vincoside and isovincoside (strictosidine).

References and Notes

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(8) Since 2b and 2d are not widely resolved by PLC[6], this is a sensible conclusion, although by TLC of the corresponding pentaacetates of 2a and 2c, each epimer appeared to be pure.
(9) This research has been supported in part by grants from the NIH (CA 17127 and 00253).