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THE ROLE OF STRICTOSIDINE IN MONOTERPENOID INDOLE ALKALOID BIOSYNTHESIS

RICHARD T. BROWN, JOHN LEONARD and STEPHEN K. SLEIGH
Department of Chemistry, The University, Manchester, M13 9PL, England

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Key Word Index—*Vinca rosea*; Apocynaceae; monoterpene; indole alkaloid; biosynthesis; strictosidine; vincoside.

Abstract—In contrast to previous reports that vincoside was the sole precursor for indole alkaloids in *Vinca rosea*, the 3α epimer strictosidine has been incorporated into tetrahydroalstonine, ajmalicine, catharanthine and vindoline; the anomalous 3β to 3α inversion is no longer required.

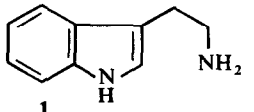
INTRODUCTION

Some years ago a series of biosynthetic investigations established the monoterpenoid glucoside secologanin (**2**) as the ultimate precursor for the C-9/C-10 non-tryptamine moiety common to the majority of indole alkaloids. Condensation of tryptamine (**1**) and secologanin (**2**) produced two diastereomeric alkaloids (**3a, b**), both found to occur in nature, but only one of which acted as a biogenetic precursor for representative alkaloids of the *Corynanthé*, *Iboga* and *Aspidosperma* types in feeding experiments with *Vinca rosea* (syn. *Catharanthus roseus*) [1]. The active isomer was considered to be the 3β epimer vincoside (**3b**), whereas the 3α epimer strictosidine (**3a**) was apparently not incorporated into these alkaloids. Hence an anomaly arose since one of the products, ajmalicine (**4a**), possessed 3α stereochemistry and inversion of 3H must occur if derived from vincoside. Further biosynthetic experiments indicated that 3H was retained throughout [1]. Although we established in biomimetic reactions that such an inversion with retention of hydrogen was feasible, the necessity for such a process when a potential precursor with the correct C-3 stereochemistry was available remained an enigma [2].

Recently we achieved biomimetic conversions of both strictosidine and vincoside into heteroyohimbine alkaloids [3]. Treatment of the former in aqueous pH 5–6.5 buffers with β-glucosidase in the presence of sodium cyanoborohydride afforded only the 3α alkaloids tetrahydroalstonine (**4b**) and ajmalicine (**4a**), whereas the latter yielded the 3β isomer akuammigine (**4c**). In parallel experiments with cyanide replacing cyanoborohydride, the 21-dehydro intermediates could be trapped out as the corresponding 21-cyano adducts (**5a**) and (**5b**) from (**3a**) and (**3b**) respectively [4]. These clear indications that strictosidine functioned as a precursor of 3α *Corynanthé* alkaloids under virtually physiological conditions led us to question the conclusions of previous biosynthetic experiments. As a consequence we have carried out our own feeding experiments with *V. rosea* and found that, contrary to previous indications, strictosidine does indeed perform a similar role *in vivo*.

RESULTS AND DISCUSSION

Tryptamine-[ar-^{3}H] was condensed with secologanin to give a mixture of strictosidine and vincoside and the latter was preferentially lactamised by treatment with triethylamine. Strictosidine-[ar-^{3}H] was then purified



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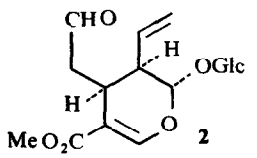

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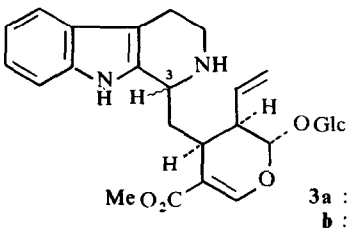
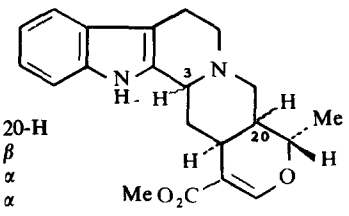
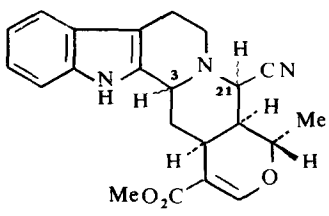


	3-H
3a	α
b	β



	3-H	20-H
4a	α	β
b	α	α
c	β	α

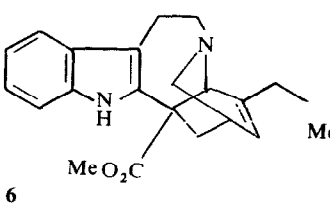


	3-H	21-H
5a	α	α
b	β	β

by Sephadex gel, ion exchange and silica chromatography, monitoring by UV and TLC, and its radiochemical homogeneity was ascertained by dilution analysis, before feeding in aqueous solution to *V. rosea* cuttings. After three days these were harvested and satisfactory incorporations were obtained into tetrahydroalstonine, ajmalicine, catharanthine and vindoline, but as anticipated none into the 3β alkaloid akuammigine (see Table 1). A previous experiment with tryptophan-[ar-^{3}H] established that although the other four alkaloids were being synthesised by the plant, akuammigine was not and hence it could be used as a 'blank' for the isolation procedures.

Table 1. Incorporations of strictosidine-[ar-^{3}H] in *V. rosea*.

Alkaloid	% Incorporation
Tetrahydroalstonine (**4b**)	0·1
Ajmalicine (**4a**)	0.2
Akuammigine (**4c**)	<0.001
Catharanthine (**6**)	0.25
Vindoline (**7**)	0.25



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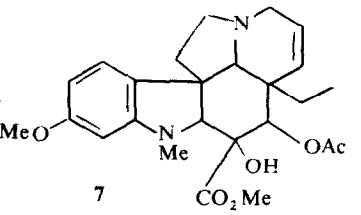

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Hence strictosidine is not only transformed into 3α *Corynanthé* alkaloids, showing that no inversion of 3-H is required, but also acts as general precursor for the *Iboga* and *Aspidosperma* types. Independent studies by Stöckigt and Zenk with *V. rosea* and other plant systems have led to the same conclusions on the role of strictosidine in indole alkaloid biosynthesis, and furthermore have shown that vincoside is apparently not utilised in *V. rosea* [5]. However in view of the ready biomimetic conversion of vincoside into 3β *Corynanthé* alkaloids, it would seem probable that vincoside will be found to have a function as an *in vivo* precursor for alkaloids with this C-3 configuration in plants where they occur and, furthermore, may also be transformed into *Iboga* and *Aspidosperma* skeleta. The alternative possibility where alkaloids derived from strictosidine would undergo inversion of H-3 from α to β seems much less likely. One can envisage that there are perhaps three categories of indole alkaloid producing plants—those using strictosidine, those using vincoside and those which use both precursors. Obviously further biosynthetic work on a wider range of plants is required.

EXPERIMENTAL

Strictosidine-[ar-^{3}H]. A soln of tryptamine-[ar-^{3}H] (10 mg, 0.1 mCi) and secologanin (100 mg) in 0.01 M HCl (10 ml) was left under N_2 at 37° for 3 days, then evapd *in vacuo*. The residue was treated with 10% methanolic NEt_3 (10 ml), for 30 min at room temp., and again the solvent was removed. The products were then chromatographed on Sephadex LH 20 (10 g) in MeOH, fractions being monitored by UV and TLC. This removed any unreacted tryptamine and much of the secologanin. Appropriate fractions were combined and a methanolic soln loaded on to an Amberlyst A-15 acidic ion exchange column (5 g). After washing with MeOH to remove secologanin and vincoside lactam, elution with 2% methanolic HCl afforded strictosidine hydrochloride. After TLC on Si gel in $CHCl_3$–MeOH (9:1), the identity of the product was confirmed by comparison with authentic material and its radiochemical homogeneity by dilution analysis.

Vinca rosea feeding. Strictosidine-[ar-^{3}H] hydrochloride (5 μCi) was fed in aq. soln to 6 small cuttings of *V. rosea*. After 3 days the plants were macerated with MeOH (3 × 30 ml) containing 25 mg each of tetrahydroalstonine, ajmalicine, akuammigine, catharanthine and vindoline. The combined MeOH extract was evapd, the residue taken up in 2% aq. tartaric acid (100 ml) and washed with petroleum (bp 40–60°) (3 × 30 ml). After adjustment of the pH to 4·5, the aq. soln was extracted with $CHCl_3$ (4 × 30 ml), the combined organic extract dried and evapd. The 5 alkaloids were separated by prep TLC on Si gel (cyclohexane–EtOAc, 1:1) and recrystallised to constant sp. act.; ajmalicine (R_f 0.36) and tetrahydroalstonine (R_f 0.52) as their free bases from MeOH; catharanthine (R_f 0.27) and akuammigine (R_f 0.16) as their hydrochlorides from MeOH–Et_2O; vindoline (R_f 0.08) was converted to desacetylvindoline and recrystallised from EtOAc. The final sp. act. (dpm/mmol) were ajmalicine 3.0×10^5, tetrahydroalstonine 1.3×10^5, akuammigine $< 10^2$, catharanthine 3.7×10^5, vindoline 4.2×10^5.

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