Biosynthesis of Strychnine

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Summary In contrast to short-term experiments, prolonged feedings have revealed that geissoschizine (**2**) and the Wieland–Gumlich aldehyde (**3**) are specifically incorporated into strychnine (**1**) in *Strychnos nux vomica*; the detection of six alkaloids not previously found in this species is also described.

THE biosynthesis of strychnine from tryptophan and geraniol has been demonstrated in *Strychnos nux vomica* in full accord with the seco-iridoid pathway, the additional 2-carbon bridge [C(22)–C(23)] being specifically derived from acetate.[1] However the conditions for successful incorporations were found to be crucial and in fact administration of several more complex intermediates such as geissoschizine (**2**), Wieland–Gumlich (W–G) aldehyde (**3**), and diaboline (**4**) led to insignificant ($<2 \times 10^{-3}$%) incorporations into strychnine during relatively short-term hydroponic feedings both in Zürich[1] and New Haven. The problem has now been reinvestigated using the techniques of autoradiography and isotopic dilution and by following a programme of prolonged contact feedings.

Young seedlings of *S. nux vomica* were fed solutions of (±)-[3-^{14}C]tryptophan and sodium [2-^{14}C]acetate for 70—90 h at 26 °C under daylight illumination and the uptake of radioactivity into the *Corynanthé* and *Strychnos* alkaloids was assayed by t.l.c.–autoradiography. In this way the presence of geissoschizine (**2**), demethoxycarbonylgeissoschizine (**5**), and W–G aldehyde (**3**) was demonstrated and confirmed by dilution analysis (in the latter 2 cases), conversion into the corresponding alcohols with KBH_4, and crystallisation to constant specific activity (Table 1). No radioactivity was ever detected in diaboline (**4**) (*N*-acetyl W–G aldehyde) in our dilution experiments.

We next investigated the effect of replanting young seedlings after 5 days incubation with *Ar*-^{3}H labelled (**2**), (**5**), and (**3**). After *ca.* 100 days, strychnine was isolated from each feeding and crystallised to constant radioactivity; the results are shown in Table 2. In the two positive incorporations, 95—97% of the tritium label was shown to reside on the aromatic ring by permanganate degradation to dimethyl *N*-oxalylanthranilate,[2] an experiment which reveals that little or no randomisation of the ^{3}H label to other parts of the molecule had occurred.

The incorporation of geissoschizine (**2**) suggests that the pathway of strychnine biosynthesis closely parallels that of the *Strychnos* alkaloid akuammicine (**6**) in *Vinca rosea*[3] where it is the methoxycarbonyl group of (**2**) and vincoside (**12**) which is retained; in contrast, the masked aldehyde group [C(17)] of W–G aldehyde (**3**) most probably corresponds to the aldehyde function of (**2**). The isolation but non-incorporation of geissoschizal (**5**) in *S. nux vomica* is indicative of the intervention of dehydro-preakuammicine (**7**) which serves as a pivotal intermediate for both akuammicine (**6**) and strychnine; *i.e.* the C(1) unit is lost after rearrangement of the *Corynanthé* to *Strychnos* skeleton. The isolation of detectable amounts of condylocarpine (**8**) in our seedlings further affirms this duality. Geissoschizal (**5**) even if not directly related to the biosynthesis of (**1**) might still be the progenitor of normacusine B (**9**), also isolated from the young *S. nux vomica* seedlings.

The successful incorporation of the W–G aldehyde (**3**) confirms the earlier postulates by Woodward[4] and Robinson,[5] but the failure either to detect or to incorporate diaboline (**4**) suggests that the biosynthesis after (**3**) may involve *C*-alkylation with acetic acid to the aldol acid (**10**).[5] Experiments 4 and 5 support this hypothesis (Table 1). In

TABLE 1. *Incorporation into alkaloids of* 1—2 *months old* Strychnos nux vomica

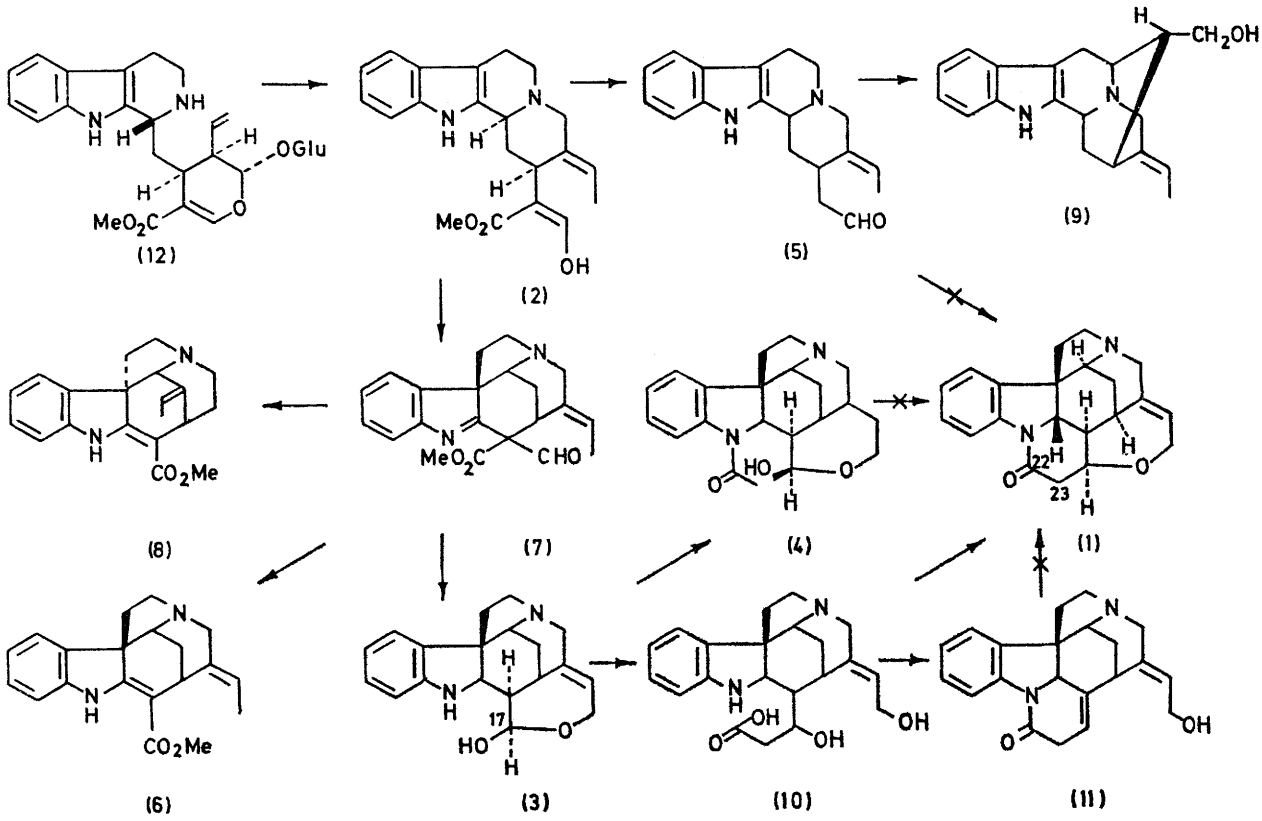
Expt.	Precursor	Feeding time (h)	% Incorporation into alkaloids[a] Strychnine (**1**)	W-G Aldehyde[b] (**3**)	Diaboline[b] (**4**)	Geissoschizal[b] (**5**)	Prestrychnine'[c] (**10**)
1	(±)-[3-^{14}C]Tryptophan	66	0·024	0·10	0·0005	—	—
2	(±)-[3-^{14}C]Tryptophan	72	0·040	—	0·0004	0·096	—
3	Sodium [2-^{14}C]acetate	66	0·032	—	0·0002	—	—
4	Sodium [2-^{14}C]acetate	67	0·0095	—	—	—	0·024
5	(±)-[3-^{14}C]Tryptophan	92	0·0041	—	0·0004	—	0·038

[a] (Total d.p.m. in alkaloid × 100%)/(total d.p.m. fed). [b] Assayed as respective alcohols after KBH_4 reduction. [c] Assayed by warming strychnine-free acidified fraction, followed by dilution with cold strychnine.

TABLE 2. *Incorporation* (%) *of alkaloids into strychnine*

Precursor	Time of feeding (days)	Growth period (days)	Strychnine	% ^{3}H on the aromatic ring
[*Ar*-^{3}H]Geissoschizine (**2**)	4·9	98	0·23	97
[*Ar*-^{3}H]Geissoschizal (**5**)	5·5	117	0·002	—
[*Ar*-^{3}H]W-G Aldehyde (**3**)	5·0	124	1·60	95

these two experiments, the initial plant extracts (in $CHCl_3$) were washed once with dilute Na_2CO_3 before the alkaloidal extractions were performed. The basic aqueous fraction contained a base-soluble alkaloid (amino-acid); this was converted by warming in dilute acid into strychnine which after dilution with inactive strychnine possessed several times the activity of the strychnine obtained by 'normal' work-up. In fact, by comparison of the incorporations of experiments 4 and 5 with those of experiments 3 and 2 respectively, the incorporations that we previously measured for strychnine during a 'normal' work-up appear to be the sum of the activities of biosynthesised strychnine and the amino-acid ('prestrychnine') which reverts to (**1**) during acid treatment. The plausible structure (**10**) suggested for 'prestrychnine' gains support from the isolation of large amounts (*ca.* 5 mg/100 g roots) of isostrychnine (**11**) from the roots (but not the stems) of young seedlings. The details of these final stages are currently under investigation.



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