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BIOSYNTHETIC RELATIONSHIP BETWEEN INDOLE ALKALOIDS PRODUCED BY CELL CULTURES OF *AILANTHUS ALTISSIMA*

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ABSTRACT.—Feeding experiments in which ^{14}C-tryptophan was administered to cell cultures of *Ailanthus altissima* showed that the biosynthetic sequence of the produced alkaloids is: tryptophan (**1**)→β-carboline-1-propionic acid (**2**)→4,5-dihydrocanthin-6-one (**3**)→canthin-6-one (**4**)→1-hydroxy-canthin-6-one (**5**)→1-methoxy-canthin-6-one (**6**)→1-methoxy-canthin-6-one-3-oxide (**8**). 2-Hydroxy-canthin-6-one (**9**), 4-hydroxy-canthin-6-one (**11**), and 5-hydroxycanthin-6-one (**10**) derive from **4**, but their methoxyderivatives were not detected in our cultures. Canthin-6-one-3-oxide (**7**) also derives from **4** but is not further transformed. ^{14}C-Tryptamine was ineffective in labelling the alkaloids. Except for canthin-6-one (**4**) and 1-methoxy-canthin-6-one (**6**), none of the above alkaloids has ever been described in plant cell cultures, and 4,5-dihydrocanthin-6-one (**3**), 2-hydroxy-canthin-6-one (**9**), and 4-hydroxy-canthin-6-one (**11**) have never actually been found in nature.

To date about 100 alkaloids with β-carboline structure have been found in higher plants and microorganisms. Despite the fact they are among the simplest indole alkaloids and are present in a large number of plants (1), very few papers have been published about their biosynthetic pathways (2-5). It has been demonstrated that tryptophan is their common precursor, but the structure of the second block of their molecule and the biosynthetic steps are largely assumptions. This is mainly because of the difficulties of feeding experiments with intact plants.

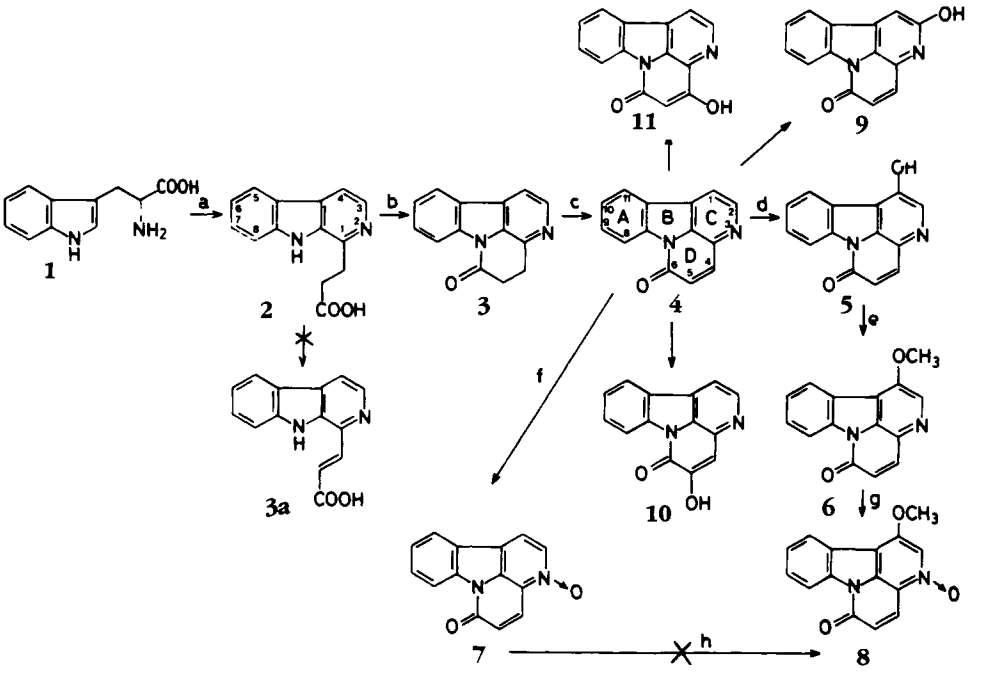
In our laboratories we have obtained cell cultures of *Ailanthus altissima* Swingle (Simaroubaceae), a producer of large amounts of β-carboline alkaloids, mainly canthin-6-one (**4**) and 1-methoxy-canthin-6-one (**6**) (7). These cell cultures are useful for studying the biosynthesis of the alkaloids, particularly the sequence of the steps involved.

In a first series of experiments the cultures were fed ^{14}C-tryptophan. The various alkaloids produced were extracted and isolated according to their tlc radiochromatographic peak, then purified and identified by comparison with the pure alkaloids (7). In a second series of experiments, after purification, each single labeled alkaloid was administered to new cultures. The crude extracts of the alkaloids produced by these second cultures were analyzed by tlc and radiochromatographic scanning.

All the tlc analyses were similar in their alkaloid composition, but their radiochromatographic pattern differed according to the administered labeled precursor. All the alkaloids showing a tlc radioactivity peak derived from the labeled compound administered. The unlabeled compounds evidently occupy a position in the biosynthetic sequence that precedes that of the radioactive compound administered. It was thus possible to place each compound produced in its proper position along the sequence reported in Scheme 1.

The isolation of **3** from *A. altissima* cultures has already been reported (7). It might be derived from **2** in two different ways: via 4,5-dihydro-canthin-6-one (**3**) or via β-carbolyl-1-acrylic acid (**3a**), in which the oxidation in positions 4 and 5 occurs, respectively, before and after the closure of ring D in molecule **4**. These two compounds have never been found in nature so far, but both can be obtained by chemical manipulation of **4** (6).

In our laboratory compounds **3** and **3a** were prepared from **4** and their chromato-



SCHEME 1. Biosynthetic sequence of the alkaloids produced by cell cultures of *Ailanthus altissima*

graphic behavior and spectroscopic characteristics determined. In order to find them, even if only in traces, a batch of 50 liters of cell suspension cultures was processed. Only **3** was found; **3a** could not be detected, but the cultures were found to contain a series of minor alkaloids such as 4-methoxy-β-carbolyl-1-carboxylic acid methyl ester, 1-hydroxy-canthin-6-one (**5**), and 5-hydroxy-canthin-6-one (**10**), not previously isolated from cell cultures, and 2-hydroxy-canthin-6-one (**9**) and 4-hydroxy-canthin-6-one (**11**), not yet found in nature (7).

RESULTS AND DISCUSSION

The aim of a first series of experiments was to obtain labeled metabolites. Three different experiments (A,B, and C) were performed as reported in Table 1. Large amounts of ^{14}C-tryptophan were added to 60 ml of 10- to 13-day *A. altissima* cultures. The age at which to make this addition was chosen on the basis of the culture growth, which differed from one batch to another. In experiments A and B, D,L-trytophan was added, but in Experiment C, in the hope of obtaining intermediates with greater specific activity, L-tryptophan was used.

Unexpectedly, the radioactivity distribution was the same in all three experiments. In experiments A and B the sum of the radioactivity in the residual- extracted-cells (attributable to tryptophan taken up into proteins) and in the toluene extracts (tryptophan taken up in alkaloids) as more than 50%, indicating that the dextrorotatory form had also been used.

In experiment D, tryptamine was added in place of tryptophan, and no uptake was seen in the alkaloids. The tryptamine was probably absorbed by the cells, as indicated by the low radioactivity in the supernatant, and, in fact, was all recovered in the toluene and BuOH extracts. Chromatographic analysis of these extracts showed the radioactivity was entirely accounted for by nonmetabolized tryptamine. It is tempting to attribute this result to the low alkaloid production noted in this experiment, but experi-

TABLE 1. Incorporation of Radioactivity in *Ailanthus altissima* Cell Cultures

Experiment	Age of cultures (days)	Administered product	μCi/flask (μg)	alkaloid production[a] (mg/flask)	Radioactivity present expressed as percent of the administered amount				
					Culture medium	Cells			
						Toluene extracts[b]	*n*-BuOH extract[b]	Residual	
								H_2O	Cells
A	10+3[c]	D,L-[methylene-^{14}C]-tryptophan	78 (285)	8.6	22	14	35	6	23
B	13+2	D,L-[methylene-^{14}C]-tryptophan	64 (234)	10.9	13	11	26	5	36
C	11+2	L-[methylene-^{14}C]-tryptophan	100 (355)	2.6	16	14	38	4	32
D	11+2	[methylene-^{14}C]-tryptamine	100 (304)	2.9	11	35	44	5	—[d]
E	12+1	[^{14}C]-**2**	0.60 (70)	5.7	44	12	36	8	—[d]
F	13+1	[^{14}C]-**3**	2.51 (4,514)	7.2	49	31	17	3	—[d]
G	8+10	[^{14}C]-**4**	11.04 (6,690)	7.6	13	36	42	9	—[d]
H	15+6	[^{14}C]-**7**	0.50 (769)	2.8	36	51	13	—[d]	—[d]

[a]Determined as canthin-6-one.
[b]See Experimental.
[c]Time of contact of the labeled product.
[d]No radioactivity.

ment C, which was run in parrallel and gave similar production, showed good tryptophan uptake, so this hypothesis must be excluded.

Total alkaloid production in experiments A and B was about 10 mg, in agreement with the control culture treated only with the vehicle. Experiment C produced only about a quarter as much, probably because the cultures were harvested just at the beginning of the production phase. Every 24 h a sample of 0.1 ml of broth was tested for radioactivity, and it was clear that after the first day of contact more than half the precursor had already been transferred into the cells. The cultures were harvested after 2-3 days when the medium contained only traces of alkaloids, and radiochromatographic and electrophoretic analyses indicated the radioactivity present (13-22%, see Table 1) was mainly due to polar products different from tryptophan. The cells were treated with EtOH and the extract was analyzed by radiochromatography. A typical radiochromatographic pattern of the labeled alkaloids is shown in Figure 1.

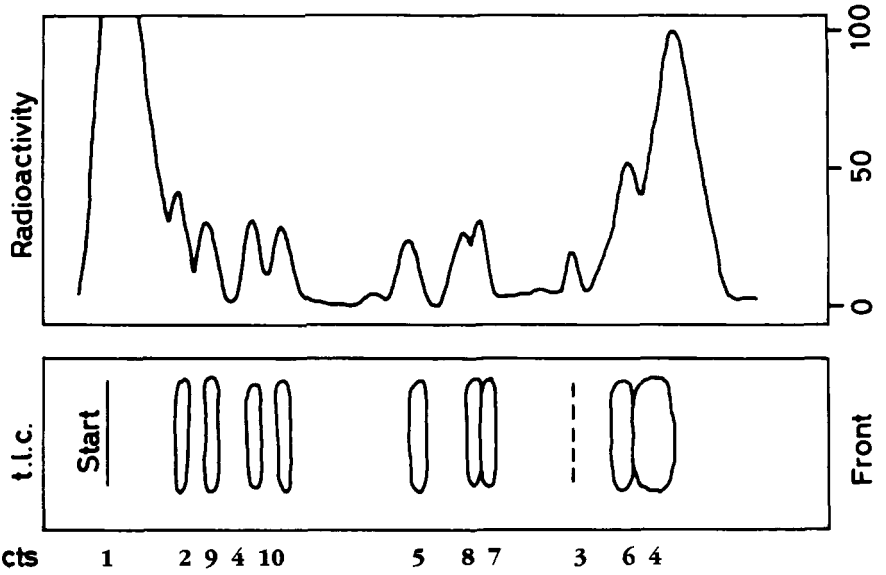


FIGURE 1. Radiochromatographic pattern of the EtOH extract of Experiment A (Table 1) where ^{14}C-tryptophan was added. Tlc in system c (see Chromatographic procedures).

The EtOH extract was partitioned with toluene, *n*-BuOH, and H_2O, as described in Experimental, to separate the metabolites according to their polarity. The toluene extract contained about 90% of the total produced alkaloids, so the 11-14% radioactivity it contained was indicative of uptake of tryptophan into the alkaloids mainly cathin-6-one (**4**), 1-methoxy-canthin-6-one (**6**), and 3-oxides **7** and **8** with traces of **3**. In all three experiments, **4** accounted for at least 70% of the total alkaloids.

The butanolic extract contained about one-third of the administered radioactivity. The alkaloids present were **2** and much smaller amounts of **9**,**10**, and **11**. The majority of the radioactivity was due to products which showed a very low tlc Rf in all the solvent systems employed and had spectra typical of indole derivatives. These products may be precursors close to tryptophan, with their C ring open and/or the carboxylic group still present. This was supported by the fact that they move into *n*-BuOH from an aqueous acidic solution. No tryptophan was present in this fraction.

The residual H_2O solution, exhausted by toluene and butanolic extractions, contained only 5% of the radioactivity and was not further processed. Finally, a large amount of radioactivity was found in the extracted cells and determined by combustion. This fraction can be ascribed to tryptophan incorporated into the cell proteins.

No labeled CO_2 was formed in experiments A,B and C. The labeled alkaloids in the toluene and BuOH extracts of Experiments A, B, and C were purified by tlc in one or more solvent systems until their specific activity was constant. The values are reported in Table 2.

TABLE 2. Specific Activity of the Alkaloids from Cell Cultures of *Ailanthus altissima* Fed Labeled Precursors as Reported in Table 1

Experiment	Administered Product	Specific Activity of Alkaloids Produced[a]						
		2	**3**	**4**	**5**	**6**	**7**	**8**
A	D,L-[methylene-^{14}C] tryptophan	4.76	n.d.[b]	1.65	0.47	0.19	n.d.	n.d.
B	D,L-[methylene-^{14}C] tryptophan	3.05	n.d.	0.55	n.d.	0.14	n.d.	n.d.
C	L-[methylene-^{14}C] tryptophan	13.00	n.d.	5.30	n.d.	n.d.	n.d.	n.d.
D	[methylene-^{14}C] tryptamine	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
E	[^{14}C]-**2**		n.d.	0.123	n.d.	n.d.	n.d.	n.d.
F	[^{14}C]-**3**	—[c]		0.139	n.d.	0.031	n.d.	n.d.
G	[^{14}C]-**4**	—[c]	—[c]		0.466[d]	0.140	0.051	n.d.
H	[^{14}C]-**7**	—[c]	—[c]	—[c]	—[c]	—[c]		—[c]

[a]Data are expressed as μCi/mg.
[b]Low radioactivity evident on tlc but specific activity not determinted.
[c]No radioactivity on tlc.
[d]Specific activities of other analogous hydroxy-canthin-6-ones, not reported in this Table, were 0.133, 0.131, and 0.129 for **9, 10,** and **11**, respectively.

Some minor alkaloids were radioactive on radiochromatographic scanning, but the amounts were too small to determine their specific activity. These cases are reported as n.d. (not determined) in Table 2. The specific activities are in agreement with batch of orgin, the age, and the different phase of production of each culture.

The specific activities of the same products were of the same order of magnitude in Experiments A and B. The slightly higher values in A could be explained by the lower production and the larger dose of radioactivity administered. Obviously, the specific activity of the alkaloids isolated was four times higher in experiment C where production was only about one quarter of that of A, and the incorporation of labeled tryptophan was the same (see toluene extract in Table 1).

The specific activity values in each experiment clearly suggest how biosynthesis proceeds. Every new labeled molecule, when formed, is diluted with the existing unlabeled ones, and, consequently, the specific activity drops at each step. This helps in establishing whether a compound was synthesized before or after another, but does not explain whether it is actually part of the sequence starting from **1** and ending with **8**, or a side product.

In order to assess the real position of each alkaloid, a second series of experiments was performed, feeding cultures with each labeled alkaloid isolated in the experiments A,B, and C, (Exps E,F,G, and H in Tables 1 and 2). The radiochromatographic pattern of the extracted alkaloids, revealing the presence or absence of radioactivity for each product, and the determination of their specific activity when possible, make it possible to determine each alkaloid's position in the biosynthetic sequence in Scheme 1:

Step **a**.—Compound **2** was produced quickly and in our conditions did not proceed from tryptamine. ^{14}C-Tryptamine, in fact, did not label any of the alkaloids produced (Experiment D, Table 2). The presence of **2** indicated that the ring C of the β-carboline structure is already complete when the D ring is still open. The origin of the 4-C fragment in position 1 of **2**, probably deriving from the tricarboxylic acid cycle, is under investigation. Radiochromatographic analysis of butanolic extracts indicated a large amount of radioactivity in peaks corresponding to unidentified products more polar

than **2** (see Figure 1). Thus, either there are several other steps between **1**and **2**, or side products are formed. Compound **2** was present only in traces in the cultures but, as expected because of its position close to tryptophan in the biosynthetic pathway, its very high specific activity made tlc detection far easier.

Step **b**.—Labeled **2** was recovered from the BuOH extracts of experiments A,B, and C, purified in tlc, and identified by uv and ms analysis. The total amount of **2** was 73 μg with specific activity of 8.6 μCi/mg. As it was so scarce, the labeled **2** was all fed to a 20 ml 12-day-old culture (Table 1, Experiment E). Twenty-four h later the culture was harvested. The alkaloids extracted with toluene were 5.71 mg expressed as **4**. Compound **3** was not detected. Canthin-6-one (**4**), isolated and purified by tlc, had specific activity of 0.123 μCi-mg and incorporated 11% of the total administered radioactivity. The dramatic drop of radioactivity was explained by the small amount of **2** added, by the extensive dilution of inert **4**, the main alkaloid produced, and by the fact that at least two steps occur between **2** and **4**. However, this experiment confirmed that **2** is on the biosynthetic sequence leading to **4**.

Step **c**.—In normal cultures compound **3** was present only in traces, difficult to identify, but a feeding experiment had to be made with labeled **3** to assess whether it was an actual intermediate or a side product. Experiment F was, therefore, carried out in which labeled **3** was prepared by reduction of labeled **4** (6) obtained in Experiment B. A volume of 20 ml of a 13-day-old culture was fed with 4.5 mg of ^{14}C-**3** (specific activity 0.565 μCi/mg) and harvested 24 h later. The canthin-6-one among the extracted alkaloids (2.73 mg) was labeled with a specific activity of 0.139 μCi/mg. Incorporation of **3** into **4** corresponded to 13% of the total administered radioactivity.

Step **d**.—Compound **4** accounts for 80% of the indole derivatives produced by *A. altissima* and, therefore, it could be obtained in Exps. A and B. A 60-ml culture was supplemented with labeled **4** (see Table 1, Experiment G), and after 10 days radiochromatography showed labeled **5**, but only traces of it could be recovered. This demonstrates that oxidation occurs in position 1 when both rings C and D are completely formed.

In the butanolic extract of this experiment 2-,4-, and 5-hydroxy-canthin-6-one (**9**,**10**, and **11**) were also detected. Radiochromatographic scanning showed they were labeled and their specific activity was established. The presence of both 1-hydroxy-canthin-6-one (**5**) and 2-hydroxy-canthin-6-one (**9**) suggests that they may be formed through an epoxide intermediate (8) leading to either product as a final result. The same mechanism could be responsible for the formation of 4- and 5-hydroxy-canthin-6-one (**11** and **10**). The difference in specific activity between 1-hydroxycanthin-6-one (0.466 μCi/mg) and 2-hydroxycanthin-6-one (0.133 μCi/mg) can be ascribed to the fact that whereas the amount of former is smaller, as it is continuously depleted by conversion to **6**, the latter is diluted by the unlabeled amount stored as end product of a side pathway. The specific activities of 4-hydroxy-canthin-6-one (**11**) and 5-hydroxy-canthin-6-one (**10**) are similar (0.129 and 0.131 μCi/mg, respectively, see Table 2) both being end products of the same pathway. This explains the absence of the corresponding methoxy-derivatives among the alkaloids produced, and also why no dioxyderivative was detected in our experiments even when large volume of cultures were extracted (7).

Step **e**.—In all the experiments with tryptophan reported in Table 2 it was never possible to recover enough **5** to feed a culture, even though radiochromatography always showed a peak of radioactivity corresponding to **5**. This may be because methylation of **5** is faster than oxidation of **4**.

Most likely **6** is produced by an *O*-methyltransferase as occurs in the methylation of

the phenol groups of isoquinoline alkaloids (9). A highly specific enzyme seems to be involved in the methylation of **5**. In fact 2-methoxy, 4-methoxy-, and 5-methoxy-canthin-6-one were never detected in cultures, whereas the corresponding hydroxy-canthin-6-ones were present.

Step **f** *and* **g**.—The formation of canthin-6-one-3-oxide (**7**) and 1-methoxy-canthin-6-one-3-oxide (**8**) might be an enzymatic process. In our experiments, when cultures were quickly extracted under mild conditions (cold, at the same pH), only traces of 3-oxide derivatives were detected. Solutions of pure **4** and **6** in organic solvents turn yellow even at 4°c, because noticeable amounts of the corresponding 3-oxides form. These substances are often present in 15-day-old *A. altissima* cultures but never in new ones. It thus appeared that **7** and **8** were formed by spontaneous reactions, though an enzymatic process cannot be ruled out.

Step **h**.—Experiment H was planned to assess whether canthin-6-one-3-oxide (**7**) is a side product or an intermediate in the subsidiary pathway leading to **8** via a hypothetic 1-hydroxy-canthin-6-one-3-oxide never found in the cultures. Labeled **7** recovered from experiment B was dissolved in 0.5 ml of EtOH and fed to a 20-ml new culture (Table 2). Ten days later when the alkaloids were extracted, radiochromatography revealed that only the added **7** was labeled. Radioactivity was absent in **8** and **4**. This confirms that step **h** is not operative, and step **f** is only one-way.

Two factors are evident from Scheme 1 as a whole. First, the biosynthetic pathway from **2** to **8** is a sequence (except for methylation) of oxidative steps, in a straight line. Second, in spite of the high oxidative capacity of *A. altissima* cells, all the oxidation occurs only in the side of the molecule corresponding to positions between 1 and 5. Cell cultures of *A. altissima* produced no alkaloids bearing oxygen in the benzene structure of the indole moiety. This is in agreement with the literature (10) for the alkaloids found in *A. altissima* plants and confirms the assumption that plant cell cultures synthesize the same products as the whole plant.

The following may be an explanation why compounds **3**, **9**, and **11** have never been found in plants: compound **3** is highly sensitive to treatment with MeOH, the solvent normally used for extraction from wood. Compounds **9** and **11** are highly polar so they are hard to extract with solvents. Nevertheless, they must be present, since the methoxy-derivative of **9** has been found (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectra were measured on a Bausch & Lomb Spectronic 2000 spectrophotometer. Radioactivity was measured with a Packard-Tricarb mod. 4530 spectrometer. Radioactivity peaks in tlc plates were detected with a radiochromatoscanner Packard model 7201.

LABELED COMPOUNDS.—DL-[methylene^{14}C]-tryptophan, purchased from Amersham (G.B.), specific activity 274 μCi/mg, was used in Experiments A and B; L-[methylene-^{14}C]-tryptophan, purchased from Amersham, specific activity 282 μCi/mg, used in Experiment C; [methylene-^{14}C]-tryptamine, from New England Nuclear, specific activity 329 μCi/mg, was used in Experiment D.

PLANT MATERIAL.—Callus cultures were obtained from leaves, stems, and shoots of *A. altissima*, cut into small pieces, and sterilized by immersion in an 0.5% solution of $HgCl_2$ for at least 1 min. After transfer to solid Murashige and Skoog's (M.S.) medium (12) the 3-week-old-calluses were harvested and maintainance subcultures prepared on M.S. supplemented with 0.1 ppm of naphthalene acetic acid (NAA) and 1.0 ppm of benzyladenine (BA). When submerged liquid cultures were needed, these were obtained by transferring the callus cells into 300-ml Erlenmeyer flasks with 60 ml of M.S. medium supplemented with 1 ppm of 2,4-dichloro-phenoxyacetic acid (2.4-D) and stirred on a rotary shaker (100 rpm) at 28° in the dark.

ADMINISTRATION OF LABELED PRECURSORS.—In experiments A, B, C, and D labeled tryptophan or tryptamine were dissolved in 0.5 ml of distilled H_2O, filtered on a Millex-HA (Millipore) to sterilize,

then administered aseptically to a 60-ml cell suspension culture. In experiments E, F, G, and H 20 ml of cell suspension culture grown in 100-ml conical flasks were fed with the labeled precursor dissolved in 0.5 ml of EtOH. The feeding times for each experiment are set out in Table 1. Parallel tests using EtOH alone indicated that the EtOH did not affect production.

ALKALOID EXTRACTION.—Cell suspension cultures were centrifuged at 5000 rpm as soon as harvested. The culture broth was only analyzed for its radioactivity and alkaloid content. It contained so little alkaloids that it could be overlooked. The cell material was suspended in 80% EtOH (100 ml), homogenized with an ultraturrax apparatus, and filtered by suction. The extraction was repeated twice. The extracts were pooled, and the alkaloids were determined spectrophotometrically as canthin-6-one (13).

Labeled alkaloids were detected by radiochromatographic scanning of pooled ethanolic extracts on tlc plates in solvent systems b and c (see below). Pooled ethanolic extracts were concentrated under low pressure to one tenth of the initial volume, then brought to pH 8 with NH_3 and extracted twice with 20 ml of toluene. The pooled toluene extracts were dried over Na_2SO_4, reduced to small volume, and the alkaloids **3, 4, 6, 7,** and **8** were separated by column chromatography and on tlc plates in systems b and c. The uv spectrum was used for identification and, when possible, they were analyzed by ms and nmr against pure standards (7). The aqueous layer after toluene extraction was brought to pH 3 and extracted three times with *n*-BuOH saturated with H_2O. The pooled butanolic extracts were concentrated under low pressure, and the alkaloids **2, 5, 9, 10,** and **11** were separated on analytical tlc plates with solvent system c.

The alkaloids on the tlc plates were visualized in uv light, scraped off, eluted with CH_2Cl_2-MeOH (1:1), purified in different solvent systems and, when possible, the specific activity was determined.

CHROMATOGRAPHIC PROCEDURES.—Tlc was performed on silica gel plates using the following solvent systems: (a) CH_2Cl_2-iPrOH (92:8), (b) *n*-hexane-Me_2CO (60:40), (c) CH_2Cl_2-MeOH (80:20).

Column chromatography.—Alkaloids in the toluene extract were first partitioned by column chromatography. A silica gel G-60 column (1×15 cm) in toluene was loaded with the concentrated toluene extract (see above) and 10 ml fractions were collected. Starting from fraction 3, the solvent toluene-EtOAc (9:1) was added, and both pure canthin-6-one (**4**) (fractions 6-9) and 1-methoxy-canthin-6-one (fractions 10-15) were eluted. By raising the EtOAc to 30%, starting from fraction 16, 4,5-dihydro-canthin-6-one (**3**) was eluted (fractions 16-18), and then with toluene-MeOH (80:20) starting from fraction 20, a mixture of the 3-oxide **7** and **8** was recovered.

All the products were purified in at least two solvent systems in comparison with authentic samples before determining their specific activity or feeding them to cultures.

PREPARATION OF LABELED ALKALOIDS.—Labeled β-carboline-1-propionic acid (^{14}C-**2**) was obtained by tlc in solvent system c from the butanolic extracts from experiments A, B, and C. The product was eluted with MeOH and purified in system a. It was identified by spectrophotometry and eims. The yield was 70 μg of **2** with specific activity 8.6 μCi/mg. Labeled 4,5-dihydro-canthin-6-one (^{14}C-**3**) was obtained from the crude ^{14}C-**4** obtained in experiment B. Compound **4** (9.1 mg) was reduced with Zn dust (6) in 0.5 ml of HOAc at 40° for 10 min. The mixture was neutralized with 1N $NaHCO_3$ loaded on an Extrelut® column, and eluted with 100 ml of CH_2Cl_2. The reaction yielded 95% of ^{14}C-**3**, purified by tlc in systems a and b and confirmed by spectrophotometry, ^{1}H nmr and eims. Its specific activity was 0.556 μC/mg. Labeled canthin-6-one (^{14}C-**4**) was obtained by chromatography on a silica gel G-60 column of the alkaloids obtained in Experiment A as reported above. Pure ^{14}C-**4** (6.7 mg) was obtained with specific activity 1.65 μCi/mg.

Labeled 1-hydroxy-canthin-6-one (**5**), 2-hydroxy-canthin-6-one (**9**), 4-hydroxy-canthin-6-one (**11**), and 5-hydroxy-canthin-6-one (**10**) were obtained from the butanolic extract of experiment G and purified by repeated tlc in systems a and c. Labeled canthin-6-one-3-oxide (^{14}C-**7**) was produced by oxidation of ^{14}C-**4** (13).

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LITERATURE CITED

1. J.R.F. Allen and B.R. Holmsted, *Phytochemistry*, **19**, 1573 (1980).
2. D. Gröger and H. Simon, *Abhandl. deut. Akad. Wiss. Berlin*, 343 (1963).
3. M. Slaytor and I.J. McFarlane, *Phytochemistry*, **7**, 605 (1968).
4. I.J. McFarlane and M. Slaytor, *Phytochemistry*, **11**, 229 (1972).
5. R.B. Herbert and J. Mann, *J. Chem. Soc. Chem. Commun.*, 841 (1980).
6. H.F. Haynes, E.R. Nelson, and J.R. Price, *Aust. J. Sci. Res.*, **A5**, 387 (1952).

7. N. Crespi-Perellino, A. Guicciardi, G. Malyszko, A. Minghetti, M. Ballabio, and B. Gioia, *J. Nat. Prod.*, (in press).
8. P. Manitto, in: "Biosynthesis of Natural Products," Ellis Horwood, Chichester, 1981, p. 90.
9. S. Muemmler, M. Rueffer, N. Nagakura, and M.H. Zenk, *Plant Cell Rep.*, **4**, 36 (1985).
10. T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, **32**, 170 (1984).
11. G.A. Cordell, M. Ogura, and N.R. Farnsworth, *Lloydia*, **41**, 166 (1978).
12. T. Murashige and F. Skoog, *Physiol. Plant*, **15**, 473 (1962).
13. T. Ohmoto, R. Tanaka, and T. Nimaido, *Chem. Pharm. Bull.*, **24**, 1532 (1976).

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