Phytochemistry, 1977, Vol 16, pp. 561–563. Pergamon Press Printed in England.

BIOSYNTHESIS OF SECURININE, THE MAIN ALKALOID OF *SECURINEGA SUFFRUTICOSA**

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(*Revised received* 8 *November* 1976)

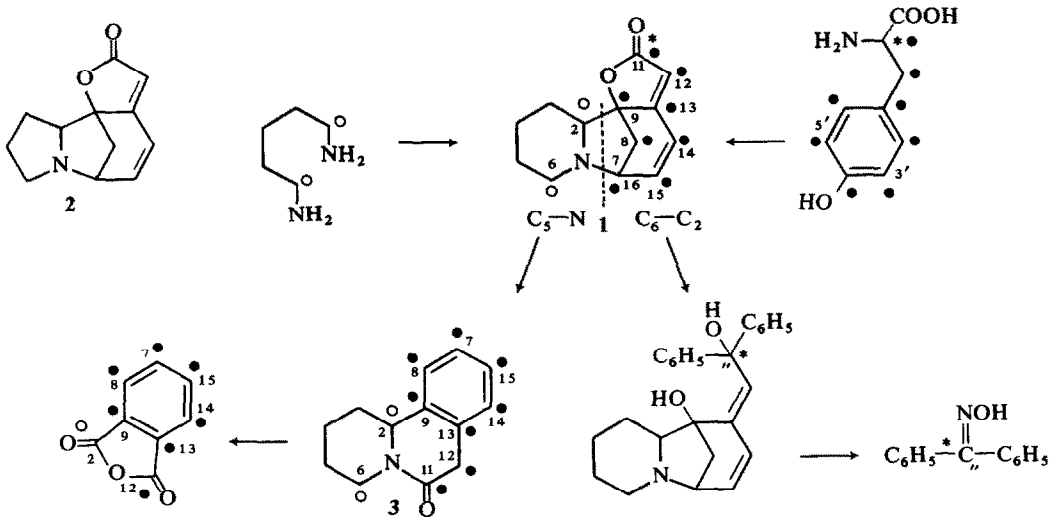
Key Word Index—*Securinega suffruticosa*; Euphorbiaceae; biosynthesis; alkaloid; securinine; tyrosine; cadaverine.

Abstract—Biosynthesis of securinine was studied by incorporation experiments in *Securinega suffruticosa*. Among presumed precursors tested, lysine, cadaverine, and tyrosine showed the highest incorporation into securinine. Degradation experiments revealed that cadaverine-[1,5-^{14}C] labelled specifically the piperidine ring of securinine and the radioactivity from DL-tyrosine-[2-^{14}C] was introduced into the C-11 lactone carbonyl. Experiments with L-tyrosine-[U-^{14}C] and L-tyrosine-[3′,5′-^{3}H; U-^{14}C] prove that the remaining C_6—C_2 moiety is derived from the aromatic ring and the C-2 and C-3 or tyrosine.

INTRODUCTION

Securinine (**1**), the main alkaloid of *Securinega suffruticosa* Rhed., has been found in both *Securinega* and *Phyllanthus* [1]; more than 15 alkaloids have been so far isolated from these plants and all, except hordenine, possess a tetracyclic structure related to securinine (**1**) or norsecurinine (**2**), its lower homologue. The unique structure of securinine makes it difficult to deduce its biogenesis by analogy with pathways of alkaloid biosynthesis, and co-occurring alkaloids, which sometimes give valuable information in biosynthetic studies, were not informative in this case. As to the origin of piperidine ring (C_5—N) of securing, it seemed reasonable to assume that this could be derived from lysine and cadaverine, by analogy with the biosynthesis of various piperidine alkaloids [2]. The presence of norsecurinine supports this view, since its pyrrolidine ring could be derived from ornithine instead of lysine, as in the case of tobacco alkaloids, in which the piperidine ring of anabasine is derived from lysine and the pyrrolidine ring of nicotine from ornithine [3].

Satoda and co-workers reported that on treatment with Zn and H_2SO_4 in absolute EtOH securinine yields a lactam (**3**) which possesses a piperidine ring and a phenyl-



acetyl residue [4]. This structure suggests that the C_6—C_2 part of securinine might arise from an aromatic amino acid. Evidence to support this hypothesis was presented in our earlier communication [5], in which we reported the specific incorporation of radioactive tyrosine and cadaverine into securinine. The present article describes

* Part 11 in the series of 'Biosynthesis of Natural Products'. For part 10 see Sankawa, U., Ebizuka, Y., Shibata, S. and Yamasaki, K. (1976) *Syôyakugaky Zasshi* **30**, 183.

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Table 1. Incorporation of ^{14}C and ^{3}H from lysine, tyrosine, phenylalanine and Na acetate into securinine

Precursors	Added amount (μCi)	Fr. wt (g)	Securinine mg	Sp. act. (dpm/mM × 10^{-4})	Total incorporation (%)
L-Lysine-[U-^{14}C]	50	76	117‡	5.3	0.026
Cadaverine-[1,5-^{14}C]	50	780	200 securinine	0.64	0.005
			325 allosecurinine	1.5	0.02
DL-Tyrosine-[2-^{14}C]	50	44	200†	1.7	0.028
L-Tyrosine-[U-^{14}C]	50	—	46	20	0.038
L-Tyrosine-[3′,5′-^{3}H; U-^{14}C]	420 $\frac{^{3}H}{^{14}C}$ = 8.4 / 50	90	70	570 $\frac{^{3}H}{^{14}C}$ = 9.8 / 58	— —
L-Phenylalanine[^{3}H-Arom]	226	2.5	0.75§	4.0	0.0003
Na-acetate-[1-^{14}C]	100	6.5*	10.6	14	0.006

* Dry weight; † 200 mg unlabelled securinine was added as a carrier; ‡ 50 mg unlabelled securine was added; § Securinine was not isolated as crystals, see Experimental.

details of our experiments carried out in the last ten years.

Two other groups have also reported on the biosynthesis of securinine. Parry showed that tyrosine-[2-^{14}C] labelled the carbonyl carbon (C-11) [6], and the pro-R proton from the β-carbon of tyrosine was retained during the course of biosynthesis [7]. Spencer and his colleagues showed that lysine-[2-^{14}C] and lysine-[6-RS-^{3}H; 6-^{14}C] were incorporated into this alkaloid and also confirmed that $^{1}\Delta$-piperideine was an intermediate [8].

RESULTS AND DISCUSSION

The results of incorporation experiments are summarized in Table 1. Sodium acetate-[1-^{14}C] was chosen to see if ornithine and acetoacetate were precursors of the C_6—C_2 moiety [9]. Higher incorporation ratios, however, were observed when radioactive lysine, cadaverine and tyrosine were administered. The plant used in the feeding experiment of cadaverine-[1,5-^{14}C] was relatively big and large amounts of securinine (200 mg) as well as allosecurinine (325 mg), the C-2 epimer of securinine, were obtained [4]. Alkaloids labelled by cadaverine-[1,5-^{14}C] were separately converted into the corresponding lactams [4], which were further oxidized with $KMnO_4$ to obtain phthalic acid. Both samples of phthalic anhydride obtained in sublimation showed approximately 50% of the activity of the original alkaloids (Table 2), indicating that cadaverine can serve as an effective precursor of the C_5—N moiety.

Recently Spenser and his colleagues showed that lysine-[2-^{14}C] and $^{1}\Delta$-piperideine-[2-^{14}C] labelled exclusively the C-2 of securinine [8]. Their results indicate that securinine belongs to the group of alkaloids whose C_5—N unit is derived from lysine in non-symmetrical fashion [2]. Although the exact positions of labelling have not been confirmed, the results obtained by Spenser's group and ours establish the origin of the C_5—N moiety.

Among the labelled compounds administered to investigate incorporation into the C_6—C_2 part, radioactive tyrosine showed higher incorporation values than phenylalanine and sodium acetate. Securinine labelled by tyrosine-[2-^{14}C] was reacted with phenyllithium. The structure of the reaction product was not investigated in detail, but its IR lacked the absorption of CO and showed the absorption due to phenyl groups. Subsequent oxidation of the reaction product afforded benzophenone, which was characterized as the corresponding oxime. The activity of benzophenone oxime, representing carbonyl carbon (C-11) of securinine, was 106% of that of securinine, indicating that all the activity was located on the carbonyl carbon (C-11). This result is well in accord with that of Parry [6].

Next, to confirm the hypothesis that all the carbon atoms of the C_6—C_2 moiety are derived from tyrosine, feeding experiments using tyrosine-[U-^{14}C] and tyrosine-[3′,5′-^{3}H; U-^{14}C] were carried out. The double labelled tyrosine was prepared by mixing tyrosine-[U-^{14}C] with tyrosine-[3′,5′-^{3}H], which had been prepared by the exchange reaction with HCl-[^{3}H] [10]. Phthalic anhydride obtained from securinine labelled by tyrosine-[U-^{14}C] showed 87% of the activity of securinine, close to that of the theoretical value (7/8 = 87.5%). Administration of tyrosine-[3′,5′-^{3}H; U-^{14}C] ($^{3}H/^{14}C$ = 8.3) yielded securinine ($^{3}H/^{14}C$ = 9.8). Since the carboxyl carbon of tyrosine is lost in the course of biosynthesis, the theoretical value for the complete retention of ^{3}H would be 8.4 × 9/8 = 9.5. This result indicates

Table 2. Distribution of ^{14}C in securinine labelled by precursors

Precursor	Securinine*	Phthalic anhydride*	Benzophenone oxime*
Cadaverine-[1,5-^{14}C]	6.4 (securinine)	3.2 (50%)	—
	15 (allosecurinine)	7.7 (51%)	
DL-Tyrosine-[2-^{14}C]	1.6	—	1.7 (106%)
L-Tyrosine-[U-^{14}C]	9.3	8.1 (87%)	

* Values are given in dpm/mM × 10^{-3}.

that the double labelled tyrosine was incorporated into securinine without loss of ^3H, and provides strong evidence that the C_6—C_2 moiety of securinine is derived from the aromatic ring, C-2 and C-3 of tyrosine, and not from other sources.

EXPERIMENTAL

Known compounds were identified by the comparison of TLC, IR and/or mmp with those of authentic samples. The plants were collected in the suburbs of Tokyo and grown in pots. Sizes and ages of the plants were not uniform.

Administration of labelled compounds Two weeks prior to the administration experiments, all leaves were removed to encourage the growth of new leaves. By this procedure feeding experiments could be carried out at any time between May and September. Labelled compounds were administered to the plants by the conventional wick method and after one week leaves were harvested to isolate alkaloid.

Extraction and isolation of securinine (**1**). Fresh leaves (780 g) harvested from the feeding experiment with cadaverine-[1,5-^{14}C] were extracted with boiling MeOH. The extracts were concentrated *in vacuo* and 5% AcOH was added to the extracts. Insoluble materials were removed by filtration and filtrate left to stand overnight to precipitate flavonoid, which was identified as rutin by NMR and IR spectral comparisons with those of an authentic sample. The solution was made alkaline with NH_4OH and extracted with $CHCl_3$ to obtain the alkaloid fraction, which was chromatographed over Si gel. A band showing strong bright yellow fluorescence in UV light (325 nm) was eluted with C_6H_6–Et_2O (2:1). Securinine (200 mg) obtained on evaporation of this fraction was repeatedly recrystallized from EtOH or *n*-hexane to constant specific activity. The fraction eluted from the column with C_6H_6–Et_2OH (1:1) afforded allosecurinine (325 mg), which was recrystallized repeatedly from *n*-hexane. In some experiments inactive securinine (50–200 mg) was added as a carrier to the alkaloid fractions. Diluted securinine was further purified by column chromatography and recrystallization as described. In the feeding experiment with phenylalanine-[^3H-arom.], securinine was not isolated and its amount was determined by UV.

Benzophenone from the phenylation product of securinine. (38 mg) **1** in Et_2O was added to the solution of PhLi prepared from Li (100 mg) and bromobenzene (1.8 g) in Et_2O. The reaction mixture was boiled under reflux until it turned blue. Excess PhLi was decomposed with dil. HCl and the Et_2O layer was extracted twice with dil. HCl. The combined HCl extracts were washed with Et_2O, made alkaline with 2N NaOH and extracted with Et_2O. Evaporation of the Et_2O gave a viscous oil, which was submitted to oxidation without any purification. It was dissolved in 10% H_2SO_4 (20 ml) containing CrO_3 (3 g) and heated at 140° in a distillation flask. Distillation was continued with occasional addition of H_2O until 100 ml of distillate was collected. The distillate was extracted with Et_2O to obtain benzophenone, which was converted into its oxime by heating with a mixture of NaOH (160 mg), H_2NOH HCl (240 mg), H_2O (4 ml) and EtOH (2 ml). Benzophenone oxime, precipitated from the concentrated and acidified reaction mixture, was collected and recrystallized from EtOH to give pure benzophenone oxime (8 mg), mp 143°.

Phthalic anhydride from securinine via lactam (**3**). **1** (200 mg) was added to a mixture of Zn powder (2 g), conc H_2SO_4 (4 g) and EtOH (10 ml). The reaction mixture was stirred for 5 hr and then neutralized with conc NH_4OH. After EtOH was removed *in vacuo*, the solution was brought to pH 9 with NH_4OH and extracted with Et_2O to obtain the lactam. Oily lactam left in a flask on evaporation of Et_2O was oxidized with 1% KOH (5 ml) and 4% $KMnO_4$ (5 ml) under reflux. 1% KOH and 4% $KMnO_4$ (each 2 ml; oxidizing solution) were occasionally added to the reaction mixture and reflux was continued for a total of 14 hr. During the reaction oxidizing solution was added 4 times. The reaction mixture was acidified with dil H_2SO_4 and $NaHSO_3$ was added to disolve MnO_2. The resulting clear soln was extracted repeatedly with EtOAc. Phthalic acid obtained on evaporation of EtOAc was sublimed to give phthalic anhydride (18 mg).

Acknowledgements—We thank Prof. I. D. Spenser for valuable discussions. Thanks are also due to Dr. S. Saitoh of Tanabe Pharm. Co. Ltd., for his generous gift of securinine, and to Mr. S. Kobayashi of the Experimental Station for Medicinal Plant Studies of this faculty for the cultivation of *S. suffruticosa*. We thank the Ministry of Education, Science and Culture for a grant-in-aid.

REFERENCES

1. Imado, S., Shiro, M. and Horii, S. (1965) *Chem. Pharm. Bull. (Tokyo)* **13**, 643. cf. Raffauf, R. F. (1970) *Handbook of Alkaloids and Alkaloid-Containing Plants.* Wiley-Interscience, New York.
2. Leistner, E. and Spenser, I. D. (1973) *J. Am. Chem. Soc.* **95**, 4715.
3. Leete, E. and Siegfried, K. J. (1957) *J. Am. Chem. Soc.* **79**, 4529.
4. Satoda, I., Murayama, M., Tsuji, Y. and Yoshii, E. (1962) *Tetrahedron Letters* 1199.
5. Sankawa, U., Yamasaki, K. and Ebizuka, Y. (1974) *Tetrahedron Letters* 1867.
6. Parry, R. J. (1974) *Tetrahedron Letters* 307.
7. Parry, R. J. (1975) *J. Chem. Soc. Chem. Commun.* 144.
8. Golebiewski, M. M., Horsewood, P. and Spenser, I. D. (1976) *J. Chem. Soc. Chem. Commun.* 217.
9. Chatterjee, A., Mukherjee, R., Das, B. and Ghosal, S. (1964) *J. Indian. Chem. Soc.* **41**, 163.
10. Kirby, G. W. and Ogunkoya, L. (1965) *J. Chem. Soc.* 6914.