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Inhibitors of Cyclic AMP Phosphodiesterase in *Picrasma quassioides* BENNET, and Inhibitory Activities of Related β-Carboline Alkaloids[1)]

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Cyclic adenosine monophosphate (cAMP) phosphodiesterase inhibitors present in *Picrasma quassioides* BENN. were identified as 1-methoxycarbonyl-β-carboline, 4,5-dimethoxycanthin-6-one and 5-hydroxy-4-methoxycanthin-6-one. The structure–inhibitory activity relationships were studied in 31 derivatives of β-carboline, 2 dimeric derivatives of β-carboline and 12 derivatives of canthin-6-one. β-Carboline derivatives with a methoxycarbonyl group and canthin-6-one derivatives with a methoxyl group generally had a strong inhibitory effect on cAMP phosphodiesterase.

Keywords——cAMP phosphodiesterase; inhibitor; Simaroubaceae; *Picrasma quassioides*; *Ailanthus altissima*; alkaloid; 1-methoxycarbonyl-β-carboline; 4,5-dimethoxycanthin-6-one; 5-hydroxy-4-methoxycanthin-6-one

Cyclic adenosine monophosphate (cAMP) phosphodiesterase inhibitors present in the roots of *Anemarrhena asphodeloides* BUNGE and the fruits of *Forsythia suspensa* VAHL have been identified as norlignans[2)] and lignans,[3)] and those in the peel of *Citrus reticulata* BLANCO, the rhizomes of *Iris florentina* L. and the roots of *Polygala tenuifolia* WILLD. as flavones,[1)] isoflavone[1)] and saponins,[4)] respectively.

This paper deals with the identification of cAMP phosphodiesterase inhibitors present in the wood of *Picrasma quassioides* BENN. and with the structure–inhibitory activity relationships in analogous compounds, such as the alkaloids present in *Picrasma quassioides* BENN. and *Ailanthus altissima* SWINGLE, and their derivatives.

Results and Discussion

The hot aqueous extracts of the wood of *P. quassioides* BENN. were fractionated into chloroform-soluble and insoluble fractions. The chloroform-soluble fraction was further fractionated into sulfuric acid-soluble and insoluble fractions. The sulfuric acid-soluble fraction showed higher inhibitory activity on cAMP phosphodiesterase than the other fractions, which indicated that the inhibitors were basic compounds. On thin-layer chromatography (TLC), this basic fraction was found to contain alkaloids, so that the fraction was chromatographed on silica gel with monitoring by TLC. The main active compounds in the sulfuric acid-soluble fraction were identified as 1-methoxycarbonyl-β-carboline (**10**), 5-hydroxy-4-methoxycanthin-6-one (**39**) and 4,5-dimethoxycanthin-6-one (**40**) which have already been isolated from this plant in our laboratory.[5,16,17)]

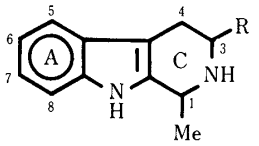
Therefore, β-carboline alkaloids isolated from *P. quassioides* BENN. and *A. altissima* SWINGLE, a plant of the same family, were tested for inhibitory activity against cAMP

TABLE I. Inhibitory Activity on cAMP Phosphodiesterase and Sources of Alkaloids Assayed for the Inhibitory Activity

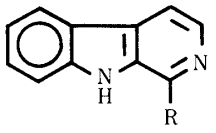
Compd. No.	IC 50 ($\times 10^{-5}$ M)	Source	References
Cf. papaverine	3.0	Commercial reagent	
1	>200	Synthetic product	6
2	30.7	Synthetic product	6
3	87.5	Commercial reagent	
4	57.5	Commercial reagent	
5	>200	*Picrasma quassioides*	5
6	24.9	*Picrasma quassioides*	7
7	9.6	Synthetic product	8
8	53.8	*Picrasma quassioides*	9
9	92.0	*Ailanthus altissima*	10
10	3.6	*Picrasma quassioides*	5
11	18.1	*Picrasma quassioides*	11
12	16.5	*Ailanthus altissima*	10
13	33.7	Synthetic product	8
14	44.8	Synthetic product	
15	22.1	Synthetic product	
16	113	Synthetic product	11
17	1.7	Synthetic product	6
18	22.9	Synthetic product	12
19	96.9	Commercial reagent	
20	69.3	Commercial reagent	
21	10.6	*Picrasma quassioides*	13
22	21.6	*Picrasma quassioides*	13
23	10.5	*Ailanthus altissima*	14
24	43.4	*Ailanthus altissima*	14
25	4.6	*Ailanthus altissima*	11
26	44.6	*Ailanthus altissima*	14
27	10.5	Synthetic product	15
28	4.9	*Picrasma quassioides*	13
29	4.9	*Picrasma quassioides*	9
30	8.0	*Picrasma quassioides*	13
31	22.7	*Picrasma quassioides*	9
32	>200	*Picrasma quassioides*	9
33	5.1	*Picrasma quassioides*	13
34	164	*Ailanthus altissima*	15
35	>200	*Ailanthus altissima*	15
36	4.8	*Ailanthus altissima*	11
37	22.3	*Simarouba amara*	19
38	70.8	*Picrasma quassioides*	13
39	1.4	*Picrasma quassioides*	16
40	10.4	*Picrasma quassioides*	17
41	12.4	*Simaba cuspidata*	18
42	>200	*Ailanthus altissima*	15
43	>200	*Ailanthus altissima*	14
44	>200	*Picrasma quassioides*	9
45	29.7	*Simaba cuspidata*	18

IC 50 is the concentration of a compound required for 50% inhibition of cAMP phosphodiesterase activity.

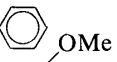
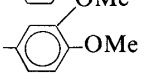
phosphodiesterase. Particularly high activity was exhibited by compound **10**, 1-(2-hydroxy-1-methoxy)ethyl-4-methoxy-β-carboline (**25**), 4,9-dimethoxy-1-vinyl-β-carboline (**28**), 1-ethyl-4,8-dimethoxy-β-carboline (**29**) and a dimer, β-carbolin-1-yl 3-(4,8-dimethoxy-β-carbolin-1-

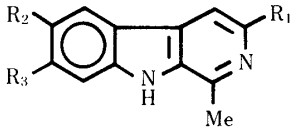


1: R=COOH
2: R=COOMe

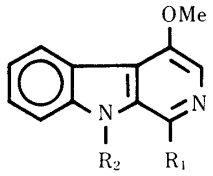


3: R=H
4: R=Me
5: R=CH_2OH
6: R=CHO
7: R=COOH
8: R=COMe

9: R=$CONH_2$
10: R=COOMe
11: R=COOEt
12: R=CH_2CH_2COOH
13: R=CH=CH- 
14: R=CH=CH- 

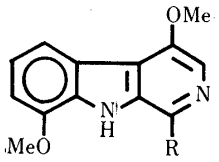


15: R_1=CH_2OH, R_2=R_3=H
16: R_1=COOH, R_2=R_3=H
17: R_1=COOMe, R_2=R_3=H
18: R_1=R_3=H, R_2=NO_2
19: R_1=R_2=H, R_3=OH
20: R_1=R_2=H, R_3=OMe

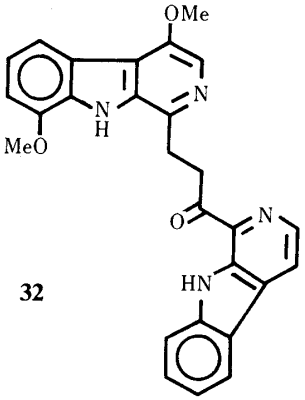


21: R_1=Et, R_2=H
22: R_1=CH=CH_2, R_2=H
23: R_1=CH_2CH_2OH, R_2=H
24: R_1=CH(OH)CH_2OH, R_2=H
25: R_1=CH(OMe)CH_2OH, R_2=H
26: R_1=COMe, R_2=H
27: R_1=COOMe, R_2=H
28: R_1=CH=CH_2, R_2=OMe

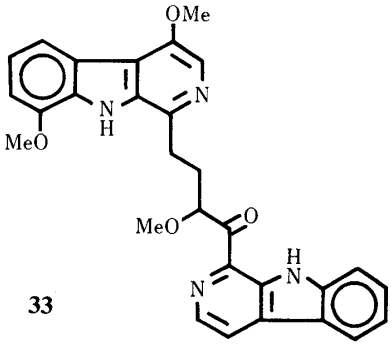
Chart 1



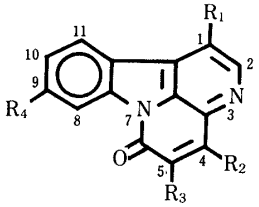
29: R=Et
30: R=CH=CH_2
31: R=CH_2CH_2OMe



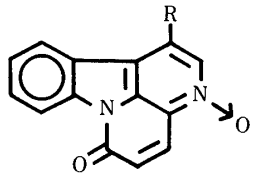
32



33

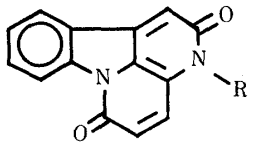


34: R_1=R_2=R_3=R_4=H
35: R_1=OMe, R_2=R_3=R_4=H
36: R_1=R_2=R_4=H, R_3=CH_2OH
37: R_1=R_2=R_4=H, R_3=OH
38: R_1=R_2=R_4=H, R_3=OMe
39: R_1=R_4=H, R_2=OMe, R_3=OH
40: R_1=R_4=H, R_2=R_3=OMe
41: R_1=R_2=R_3=H, R_4=OMe



42: R=H
43: R=OMe

Chart 2



44: R=Me
45: R=OMe

Chart 3

yl)-1-methoxypropyl ketone (**33**) among β-carboline congeners, and by 5-hydroxymethylcanthin-6-one (**36**), **39** and **40** among canthin-6-one congeners.

In addition to these alkaloids, related β-carboline alkaloids, which were donated, purchased or synthesized in our laboratory, were tested in order to elucidate the structure–activity relationships. The results are summarized in Table I.

Among β-carboline alkaloids, the compounds with a methoxycarbonyl group were more active than the corresponding compounds with methyl (**4**), hydroxymethyl (**5**), aldehyde (**6**), carboxyl (**7**) and ethoxycarbonyl (**11**) groups. 3-Methoxycarbonyl-1-methyl-β-carboline (**17**) was found to be the most potent inhibitor in this series of congeners. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**) and 3-methoxycarbonyl-1-methyl-1,2,3,4-tetrahydro-β-carboline (**2**), in which the C ring is saturated, showed lower inhibitory effects than the corresponding compounds **16** and **17**. To summarize, the inhibitory effect increased with aromatization of the tetrahydro derivatives **1** and **2**, and with the introduction of a methoxycarbonyl group at C_3 in **1** and **16**, giving **2** and **17**, respectively. In contrast to the above results, the inhibitory effect decreased with unsaturation of the ethyl moiety at C_1 (**21** and **22**, **29** and **30**). In the pairs **21**—**29** and **22**—**30** having the same substituent at C_1, it was noted that the compounds with two methoxyl groups were more active than the corresponding compounds having a single methoxy group.

Among canthin-6-one alkaloids, compounds **34**, **35**, **42**, **43** and **44**, with no substituent at C_4 and C_5, were far less active than the others. The results suggest that the presence of an oxygen atom at C_5 or at both C_4 and C_5 is essential for cAMP phosphodiesterase inhibition activity in canthin-6-one congeners.

The wood of *P. quassioides* BENN. has been extensively used as a bitter stomachic. It contains **10**, **39** and **40** as major alkaloids, which were found to have strong inhibitory activity on cAMP phosphodiesterase. The above results suggest that there may be some correlation between the therapeutic effect of *P. quassioides* BENN. and the cAMP phosphodiesterase inhibitory activity of its constituents.

Experimental

The following instruments were used for obtaining physical data. All melting points were determined with a micro-melting point apparatus and are uncorrected. The liquid scintillation counter used was an Aloka LSC-903. Silica gel 60 (Merck, precoated plate, 0.25 mm) was used for TLC and detection was achieved by illumination with an ultraviolet (UV) lamp or by spraying Dragendorff's reagent. For column chromatography, Silica gel C-200 (Wako Pure Chemical Co., Ltd.) was used. The infrared (IR) spectra were recorded with a Hitachi 295 spectrometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with JEOL JNM-4H-100 and chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; br, broad; m, multiplet). The mass spectra (MS) were measured with a JEOL JMS-01SG-2 mass spectrometer.

Samples of Medicinal Plants——Picrasmae Lignum (Japanese name Nigaki) was purchased from Uchida Pharmacy for Oriental Medicine (Tokyo). The dried wood chips were obtained from *Picrasma quassioides* BENN. collected from the Medicinal Plant Garden, School of Pharmaceutical Sciences, Toho University.

Assay Method for Inhibition of cAMP Phosphodiesterase——Samples were tested for cAMP phosphodiesterase inhibitory activity in duplicate by the method described in the previous paper.[1] All the inhibitors were added as

solutions in DMSO. The presence of DMSO in the assay medium up to 2% concentration is known to have no effect on the enzyme activity. The IC 50 value is the concentration of a compound required for 50% inhibition of cAMP phosphodiesterase activity.

Enzymes and Chemicals——Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase, cAMP, norharman, harman, harmol and harmine were obtained from Sigma, and [^{3}H]-cyclic AMP from the Radiochemical Centre. Papaverine, a reference inhibitor, tryptophan, acetaldehyde, benzaldehyde, lithium aluminium tetrahydride and 3,4-dimethoxybenzaldehyde were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). 8-Methoxycanthin-6-one and 3-methoxycanthin-2,6-dione were kindly presented by A. M. Giesbrecht of Sao'Paulo University. 5-Hydroxycanthin-6-one was kindly presented by E. V. Lassak of the New South Wales Government. The other alkaloids were all prepared in our laboratory.

Extraction and Separation of the Alkaloids——The dried commercial Picrasmae Lignum (10 g) was extracted with water (150 ml) at 90—100 °C for 6 h and the extract (1.5 g) was concentrated, frozen and dried to give a powder which was tested for inhibitory effect on cAMP phosphodiesterase at a concentration of 100 μg/ml. Each lyophilized sample was dissolved in water and partitioned with $CHCl_3$ to obtain the $CHCl_3$-soluble (0.1 g) and insoluble (1.3 g) fractions. The $CHCl_3$-soluble fraction was fractionated into H_2SO_4-soluble (0.002 g) and insoluble (0.06 g) fractions. The H_2SO_4-soluble fraction was made alkaline (to about pH 10) with 5% NH_4OH and extracted with $CHCl_3$. The extract was evaporated to dryness and used in the cAMP phosphodiesterase inhibition test.

In a large-scale extraction, dried wood chips (2 kg) of *P. quassioides* BENN. were extracted continuously with MeOH at 50 °C for 48 h. The extract was evaporated to dryness and the residue was suspended in water and extracted with $CHCl_3$. The $CHCl_3$ solution was shaken with 5% H_2SO_4. The aqueous layer was made alkaline with 5% NH_4OH and extracted with $CHCl_3$ to give a basic fraction (2.1 g). This fraction was concentrated and chromatographed on silica gel with benzene, $CHCl_3$ and MeOH as eluents: each fraction was tested for inhibitory effect on cAMP phosphodiesterase. The fractions eluted with $CHCl_3$ were found to be active, so they were further subjected to silica gel chromatography with benzene and $CHCl_3$ mixtures of various ratios as eluents.

1-Methoxycarbonyl-β-carboline (10)——The fractions eluted with $CHCl_3$ were concentrated and recrystallized from acetone to give pale yellow needles (150 mg), mp 163 °C (lit.,[7] mp 163 °C); this product was identified as **10** by comparison of the spectral data with those of an authentic sample.

5-Hydroxy-4-methoxycanthin-6-one (39)——The fractions eluted with $CHCl_3$ were concentrated and recrystallized from MeOH to give yellow needles (280 mg), mp 224—225 °C (lit.,[16] mp 223—224 °C), this product was identified as **39** by comparison of the spectral data with those of an authentic sample.

4,5-Dimethoxycanthin-6-one (40)——The fractions eluted with $CHCl_3$ were concentrated and recrystallized from acetone to give pale yellow needles (145 mg), mp 145—146 °C (lit.,[15] mp 144—145 °C), this product was identified as **40** by comparison of the spectral data with those of an authentic sample.

Synthesis of Analogous Alkaloids——1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**1**): **1** was prepared from DL-tryptophan by Snyder's method,[6] mp 255—257 °C (lit.,[6] mp 258 °C). MS *m/z*: 230 (M^+).

3-Methoxycarbonyl-1-methyl-1,2,3,4-tetrahydro-β-carboline (**2**): **2** was prepared by treatment of **1** with dry hydrogen chloride in MeOH, mp 127—129 °C (lit.,[6] mp 129—130 °C). MS *m/z*: 244 (M^+).

β-Carboline-1-carboxylic Acid (**7**): **7** was prepared by treatment of 1-(2-phenylvinyl)-β-carboline (**13**) with potassium dichromate, mp 230—232 °C (lit.,[9] mp 235 °C). MS *m/z*: 212 (M^+).

1-(2-Phenylvinyl)-β-carboline (**13**): **13** was prepared from harman (**4**) and benzaldehyde, mp 202—203 °C (lit.,[9] mp 197—199 °C). MS *m/z*: 270 (M^+).

1-[2-(3,4-Dimethoxyphenyl)vinyl]-β-carboline (**14**): **14** was prepared from **4** and 3,4-dimethoxybenzaldehyde, mp 153—155 °C. MS *m/z*: 330 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3350, 1630, 1590, 1500, 1260, 1130. ^{1}H-NMR (100 MHz, DMSO-d_6): 3.81 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 7.00—7.72 (8H, m), 8.00 (1H, s), 8.30 (1H, d, J=8 Hz), 8.36 (1H, d, J=5 Hz).

1-Methyl-3-hydroxymethyl-β-carboline (**15**): **15** was prepared by treatment of **17** with $LiAlH_4$ in THF, mp 104 °C. MS *m/z*: 212 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3500, 3200, 1610, 1360, 1240, 1010, 740. ^{1}H-NMR (100 MHz, DMSO-d_6): 2.78 (3H, s, CH_3), 4.70 (2H, d, J=4 Hz), 5.34 (1H, t, J=4 Hz), 7.07—7.60 (3H, m), 7.93 (1H, s), 8.14 (1H, d, J=8 Hz), 10.79 (1H, br s, NH).

1-Methyl-β-carboline-3-carboxylic Acid (**16**): **16** was prepared from **17** by Snyder's method,[6] mp 316—318 °C (lit.,[6] mp 305—306 °C). MS *m/z*: 226 (M^+).

3-Methoxycarbonyl-1-methyl-β-carboline (**17**): **17** was prepared by treatment of **2** with sulfur and dry xylene, mp 246—248 °C (lit.,[6] mp 245 °C). MS *m/z*: 230 (M^+).

6-Nitroharman (**18**): **18** was prepared by treatment of **4** with conc. HNO_3, mp 296—298 °C (lit.,[13] mp 299—300 °C). MS *m/z*: 227 (M^+).

Authentic Alkaloids——Authentic samples used in the tests for the evaluation of inhibitory action on cAMP phosphodiesterase had been isolated or prepared during structural studies into *P. quassioides* BENN. and *A. altissima* SWINGLE (Table I).

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