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DISCOVERY OF ANTI-INFLUENZA A VIRUS ACTIVITY OF A CORYNANTHE-TYPE INDOLE ALKALOID, HIRSUTINE, *IN VITRO* AND THE STRUCTURE-ACTIVITY RELATIONSHIP OF NATURAL AND SYNTHETIC ANALOGS

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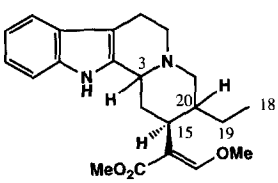
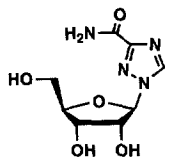
Abstract An indole alkaloid, hirsutine (**1**), was found to exhibit potent inhibitory effect against influenza A virus *in vitro*, and the essential structural feature for revealing the activity was elucidated by study of the structure-activity relationship using natural and synthetic derivatives of **1**. © 1997 Elsevier Science Ltd.

Recent studies on the development of anti-influenza virus agents have focused on the compounds having inhibitory effect on influenza virus sialidase, an enzyme which catalyses the hydrolysis of α-ketosidically-linked terminal sialic acid from glycoproteins, glycolipids, and a variety of oligosaccharide substrates.[1] The rapid emergence of drug-resistant mutants, however, makes it difficult to attain successful development of potent anti-influenza chemotherapeutants. In the course our project on the development of antiviral drugs,[2] we recently found that a plant alkaloid, hirsutine (**1**), exhibited potent anti-influenza virus activity. Hirsutine (**1**), classified as a Corynanthe-type monoterpenoid indole alkaloid, is one of the major constituents of *Uncaria rhynchophylla* MIQ.,[3] which is the original plant of the Chinese "Kampo" medicine for treatment of patients with hypertension. In this communication, we describe our preliminary findings on the inhibitory effect of hirsutine on influenza A virus *in vitro* and a study of the structure-activity relationship using natural and synthetic derivatives of this alkaloid.



3*R*, 20*R*; Hirsutine **1**
3*S*, 20*R*; Dihydrocorynantheine **2**
3*R*, 20*S*; 3-Isocorynantheidine **3**
3*S*, 20*S*; Corynantheidine **4**
3*R*, 20*R*; $\Delta^{18,19}$; Hirsuteine **5**



Ribavirin

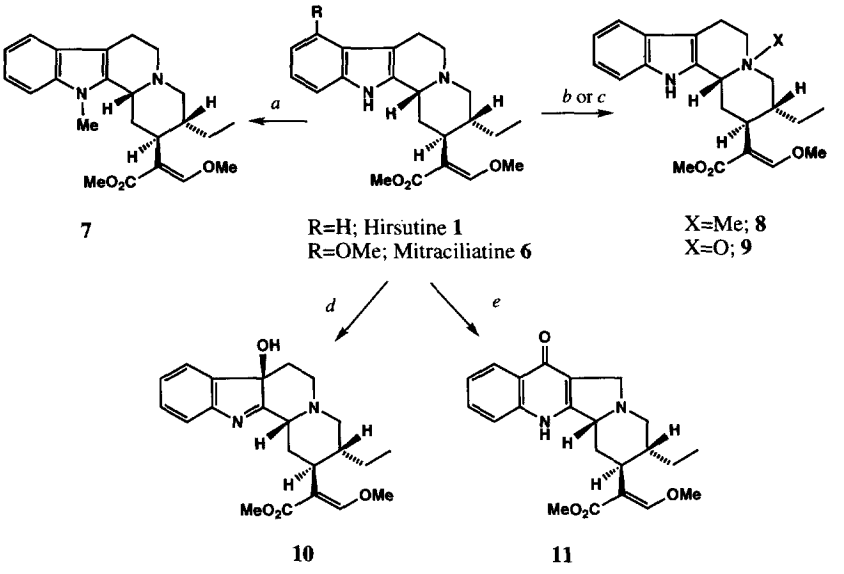
Determination of antiviral activities of the compounds against the replication of influenzaviruses was based on the inhibition of virus-induced cytopathic effect in MDCK cells.[4] Briefly, trypsinized MDCK cells were suspended in culture medium at 6 x 10^5 cells ml^{-1} and infected with virus at a multiplicity of infection (MOI) of 0.002. Immediately after virus infection, the cell suspension (50 μl) was added to each well of a 96-well round-bottomed microtitier plate (Nunclon) containing various concentration of the test compound (50 μl) and the plate was then centrifuged at 700 x g for 5 min. After 4 days incubation at 35 ℃, the number of viable cells was determined by the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method.[5] As shown in Table 1, hirsutine displayed highly potent inhibition of the replication of the strains of influenza A (subtype H3N2) (50% effective concentration (EC_{50}) = 0.4 - 0.57 μg/ml, selectivity index (CC_{50}/EC_{50}) = 41.2 - 58.8), but no activity against other subtype A (H1N1, H2N2) viruses or strains of influenza B. Compared with the activity of the clinically used ribavirin,[6] the EC_{50} of hirsutine on the subtype H3N2 is 11- to 20-fold more potent.

Table 1. Inhibitory Effect of Hirsutine and Ribavirine on the Replication of Influenza A and B Viruses

Influenza virus			50% Effective concentration (μg/ml)	
Type	**Subtype**	**Strain**	**Hirsutine 1**	**Ribavirin**
A	H1N1	PR8/34	>23.5	8.3 (>12.0)
		Bangkok/10/83	>23.5	34.2 (>2.9)
		Yamagata/120/86	>23.5	11.7 (>8.5)
	H2N2	Murakami/4/64	>23.5	2.1 (>47.6)
		Adachi/2/57	>23.5	2.2 (>45.5)
	H3N2	Ishikawa/7/82	**0.4 (58.8)**	7.9 (>12.7)
		Philippine/2/82	**0.57 (41.2)**	6.1 (>16.4)
B		Norway/1/80	>23.5	5.5 (>18.2)
		Singapore/222/79	>23.5	8.5 (>11.8)
50% Cytotoxic concentration (μg/ml)			23.5	>100

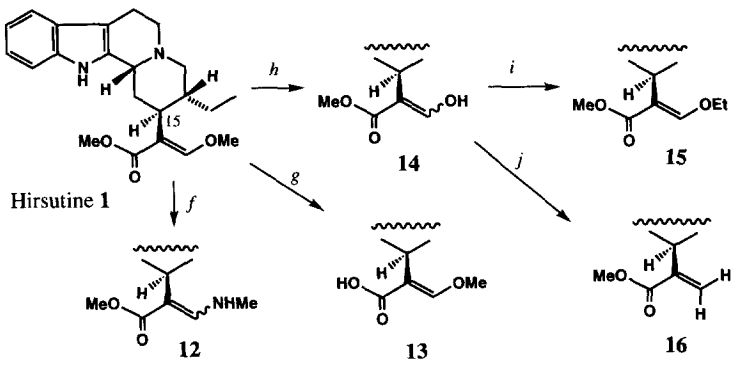
Cells: MDCK, Incubation: 35℃ 4days, Assay: MTT, ( ) : Selectivity index=CC_{50}/EC_{50}

Various derivatives of hirsutine were prepared as follows in order to find more effective compounds and to clarify the essential structural moieties in the molecule. The C-3 epimer (dihydrocorynantheine **2**),[3b] C20-epimer (3-isocorynantheidine **3**),[3b] C3,20-epimer (corynantheidine **4**),[3b] 9-methoxy derivative (mitraciliatine **6**),[7] and C18-19 vinyl derivative (hirsuteine **5**)[3b] are the naturally occurring compounds. The N_b oxide (**9**) and the N_b-methyl derivative (**8**) were respectively prepared by treatment with one equiv. of *m*-chloroperbenzoic acid or with excess methyl iodide. Modification of the indole nucleus was done by N_a-methylation with methyl iodide/sodium amide in liq. ammonia, oxidation with lead tetraacetate followed by alkaline hydrolysis, or oxidation with O_2/*tert*-BuOK in DMF to give, respectively, N_a-methylindole (**7**), 7-hydroxyindolenine (**10**), or 4-quinolone derivative (**11**). Chemical transformations of the methyl β-methoxyacrylate part were performed by the following procedures. Replacement of a methoxy group on the β-methoxyacrylate moiety to an *N*-methyl group was done by heating with methylamine in the sealed tube to afford (**12**). Hydrolysis of **1** under basic conditions (aq. LiOH) gave the acid derivative (**13**). Acidic hydrolysis of the methyl vinyl ether using HCl in acetic acid produced enol derivative



Reaction conditions: *a*, MeI, $NaNH_2$, liq. NH_3 (72%). *b*, MeI, MeOH (94%). *c*, *m*-CPBA, CH_2Cl_2. *d*, 1) $Pb(OAc)_4$, CH_2Cl_2 (63%), 2) NaOMe, MeOH (61%). *e*, O_2, *tert*-BuOK, DMF (50%).

(**14**), which was then treated with dry HCl in dry EtOH to afford β-ethoxy derivative (**15**). β-Demethoxy derivative (**16**) was prepared from **14** by a three-step operation: 1) reduction of the aldehyde with $NaCNBH_3$, 2) mesylation of the resultant primary alcohol with MsCl/pyridine in CH_2Cl_2, 3) E2 elimination using DBU in DMF. The results of the anti-virus evaluation of these compounds prepared here are summarized in Table 2. The stereochemistry of 3*R* and 20*R* configurations and the presence of an N_b-lone electron pair are essential for anti-influenza A virus activity. The N_a- or C9- substituted indole derivatives (**6**, **7**) maintain the activity, but modifications of the indole ring to hydroxyindolenine (**10**) or 4-quinolone (**11**) greatly influence the biological activity, resulting in the disappearance of the inhibitory effect. The C18-19 vinyl derivative (**5**) has almost the



Reaction conditions: *f*, aq. $MeNH_2$, heat (60%). *g*, aq. LiOH (64%). *h*, conc. HCl, AcOH (80%). *i*, dry HCl, dry EtOH (24%). *j*, 1) $NaBH_3CN$, AcOH, MeOH (85%), 2) MsCl, pyridine, CH_2Cl_2 (40%), 3) DBU, DMF (76%).

Table 2. Anti-influenza virus activity of Hirsutine derivatives

	EC_{50} (μg/ml)	CC_{50} (μg/ml)	Selectivity Index
1	0.4	23.5	58.8
2	>86.3	86.3	-
3	>44.7	44.7	-
4	>45.1	45.1	-
5	1.8	25.2	14.0
6	0.5	40.7	76.8
7	1.7	25.3	15.8
8	>100	>100	-
9	86.4	>100	>1.2
10	>100	>100	-
11	>38.1	38.1	-
12	>3.9	3.9	-
13	>39.2	39.2	-
14	>8.4	8.4	-
15	0.9	32.4	36.4
16	>62.1	62.1	-

Cells: MDCK Assay: MTT Strain: A/Ishikawa/7/82

same activity. Among the compounds modified at the substituent on C15, only the ethyl ether (**15**) exhibited the activity, indicating that the β-alkoxyacrylic acid ester function is essential for the anti-influenza A virus activity. Our preliminary findings on the potent and specific inhibitory effect on the influenza A virus of a Corynanthe-type indole alkaloid, hirsutine, which is an easily obtainable natural product, indicate the potential of this type of alkaloids as a candidate chemotherapeutic agent of influenza infection. Studies on further structure-activity relationships, the mechanism of the action, and *in vivo* experiments are in progress in our laboratories.

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