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研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

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研究テーマ 中国産 *Maackia* 属植物のルピンアルカロイドに関する化学研究

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 (有) ・ 無 (学会名・内容)

学会名：日本薬学会１１９年会（徳島）
演 題：(−)-Cytisine からの合成による (+)-hupeol の絶対配置の決定

(2) 学会誌等に発表した論文 (有) ・ 無 (雑誌名・論文名)

雑誌名：Journal of Chemical Research
テーマ：(+)-Hupeol, a possible non-basic metabolite of the lupine alkaloid (−)-cytisine in Chinese *Macckia hupehensis*

3．今後の研究計画

1．*M. amurusis* の枝から既に３種の新塩基を見出しました。それらの構造を決定し、合成検討中です。

2．入手した *M. tenuifolia* について検討する予定です。

3．(+)-Hupeol の合成により、新しい合成法として環状イミンから環状ヘミアセタールの変換反応の応用性について検討しました。その結果の一部を徳島学会で発表し、その後まとめて論文で投稿する予定です。

4．研究指導者の意見

マメ科の *Maackia* 属植物はアジアに多く分布し、日本に４種、中国には８種自生する。日本産植物が含有する塩基性成分に関する我々の研究では piperidine や quinolizidine 環を基本骨格とする通常の lupin alkaloids を主要塩基として含有するが、pyrrolidine, indolizidine 環を基本骨格とする特異な alkaloids をも含有する。このように *Maackia* 属植物は lupin alkaloids の生合成あるいは植物の化学的分類上大変興味ある植物の群であることから、未だ研究がなされていない中国産植物について成分研究を着手した。植物の採取は困難でしたが、*M. hupehensis*, *M. amurensis* および *M. tenuifolia* の３種を手にすることができた。このうち *M. hupehensis* からは(+)-hupeol のように alkaloids が塩基性窒素を含まない中性物質に代謝される最初の物質と考えられる代謝産物、典型的な lupin alkaloids の(-)-cytisine と acetamide あるいは 2-pyrrolidone の窒素がメチレン基により結合した化合物など、日本産植物には見られない興味ある物質の存在を確認している。現在、*M. amurensis* の成分を検討しており、既に３種の新塩基を見出し、これから検討する *M. tenuifolia* を含めた３種の植物の塩基性成分を明らかにすることにより、lupin alkaloids の生合成、日本産との植物分類学上の関連性など、興味ある知見が得られるものと確信している。さらに、これらの新塩基は合成によって絶対配置を含め構造を確認しているが、特に、(+)-hupeol の合成は環状イミンから環状ヘミアセタールへの一般的な変換反応として有用性が期待され、合成化学の立場からも興味ある結果を得ている。このように、王永紅君は博士の学位に充分な内容の研究を行っている。

研究指導者氏名 大宮 茂 印

5．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

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中国産 *MAACKIA* 属植物の ルピンアルカロイドに関する化学的研究

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要旨

本研究室では、マメ科植物が含有するルピンアルカロイドに関する研究の一環として日本産 *Maackia* 属植物の塩基性成分を精査し、*Maackia* 属は、通常のルピンアルカロイドに加えて、それらの基本骨格である quinolizidine あるいは piperidine 環が indolizidine あるいは pyrrolidine 環におきかわったアルカロイドを産出する特異な植物群であることが分かりました。私は、中国産 *Maackia* 属植物の塩基性成分に興味をもち、日本産との比較として、中国産 *Maackia* 属植物の一種である *M. hupehensis* について検討し、三つの新塩基性成分の構造を決定したうえで人工合成も成功したことについて報告します。

Key word index — *Maackia hupehensis*, leguminosae, lupin alkaloids, (-)-*N*-(2-oxopyrrolidinomethyl)cytisine, (-)-*N*-(*N*-acetylaminomethyl)cytisine, (+)-hupeol, (-)-cytisine, (-)-*N*-methylcytisine, (-)-lusitanine, (-)-epibaptifoline.

Abstract - Three new lupine alkaloids, (-)-*N*-(2-oxopyrrolidinomethyl)cytisine (**1**), (-)-*N*-(*N*-acetylaminomethyl)cytisine (**2**) and (+)-hupeol (**3**), were isolated together with 9 known alkaloids from Chinese *Maackia hupehensis*, which is grown in the southern China. The alkaloidal constituents of *M. hupehensis* was shown to be comparable to those of the southern species of Japanese *Maackia* plants. The Chinese *Maackia* plant also showed the relationship between geographical distribution of the plant and structural type of alkaloids contained in the plant, similarly to the relationship observed in Japanese species.

INTRODUCTION

The structural types of lupine alkaloids which occur in Japanese *maackia* plants (four species, *M. amurensis*, *M. tashiroi*, *M. floribunda* and *M. floribunda f.pubescens*) are related to geographical distribution of the plants[1]. The plant of *M. amurensis* is distributed in the north of Japan. It accumulates sparteine-type lupine alkaloids such as (+)-sparteine and (-)-lupanine and does not lupinine-type alkaloids such as (+)-epilupinine, (+)-tashriomine and (-)-lusitanine[1, 2]. The plants of *M. tashiroi*, *M. floribunda* and *M. floribunda f.pubescens* are growing in the south area of Japan, and the main constituents of those plants are lupinine-type alkaloids and no sparteine-type is found[1,3]. Furthermore the Japanese *Maackia* plants occur unusual type of lupine alkaloids, such as (-)-camoensidine[4] , (+)-tashiromine[3] and (+)-maackiamine[2], which contain a pyrrolizidine or an indolizidine ring, together with common lupine alkaloids which consist of a piperidine or a quinoliziding ring. The cytisine- and anagyrine-type alkaloids are constituents common to plants of the two groups. These phenomena are interesting from the viewpoints of chemotaxonomy of leguminous plants and biosynthesis of lupine alkaloids. This report describes the isolation and structural determination of eleven alkaloids, in which (-)-*N*-(2-oxopyrrolidinomethyl) cytisine (**1**) , (-)-*N*-(*N*-acetylaminomethyl)cytisine (**2**) and (+)-hupeol (**3**)[5], were new alkaloids and also describes a comparison of alkaloidal constituents of *M. hupehensis* with those of Japanese *Maackia* plants.

RESULTS AND DISCUSSION

The alkaloid mixture (5.4g) obtained from 75% methanol extracts of the air dried stem (1.2kg) of *M.hupehensis*, collected in Jiang xi province, China, in May, was repeatedly chromatographed on silica gel columns to give eleven lupine alkaloids, (-)-*N*-(2-oxopyrrolidinomethyl)cytisine (**1**), (-)-*N*-(*N*-acetylaminomethyl)cytisine (**2**), (+)-hupeol (**3**), (-)-cytisine (**4**, 25%), (-)-methylcytisine (**5**, 5%), (-)-*N*-formalycytisine (3%), (-)-epibaptifoline (21%), (-)-lusitanine (12%), (+)-epilupinine (3%), (-)-*N*-(3-oxobutyl)cytisine (trace), and(-)-rhombifoline (trace). in which **1**, **2** and **3** were new alkaloids . The known alkaloids were identified by comparing directly with authentic samples in all measurable respects (mass spectrometry, ^{1}H-NMR, IR, $[\alpha]_D$, co-TLC and HPLC) as described in our previous paper [6].

The total base (1.3g) obtained from the air dried leaves (750g) was similarly treated to give seven known alkaloids, (-)-cytisine (**4**, 16%), (-)-methylcytisine (**5**, 5%), (-)-*N*-formalycytisine (2%), (-)-epibaptifoline (15%), (-)-lusitanine (11%), (+)-epilupinine (4%) and (-)-lupinine (1%). This is the first example of the coexistence of (+)-epilupinine and (-)-lupinine in plant of genus *Maackia*.

Alkaloid **1** gave colorless crystals from $CHCl_3$, mp 169 ~ 170^0 C, and alkaloid **2** was isolated as an oily compound. The molecular formula of **1** and **2** were established by a high resolution mass spectra to give $C_{16}H_{21}N_3O_2$ (*m/z* 287.1627, calc. 287.1625) and $C_{14}H_{19}N_3O_2$ (*m/z* 261.1473,

calc.261.1478), respectively. The mass spectra of **1** and **2** both revealed the prominent fragment ions at *m/z* 203, 189, 160 and 146 which are characteristic of the type of *N*-alkylcytisine like (-)-*N*-(3-oxobutyl)cytisine [6, 7]. The ^{1}H and ^{13}C-NMR ($CDCl_3$) of **1** and **2**, which were assigned by analysis of ^{1}H -^{1}H COSY and ^{1}H -^{13}C COSY spectra, also resembled that of (-)-methylcytisine(**5**), as shown in Table 1 and 2. These results suggested that new alkaloids **1** and **2** might be *N*-substituted cytisine.

The presence of the isolated methylene group in the molecule of **1** was presumed from the two doublets, which were coupled only with each other, at δ3.91 (1H, d, J =12.2Hz) and δ3.77 (1H, d, J=12.2Hz) in the ^{1}H-NMR of **1**. The signals at δ175.9 (s), δ31.2 (t), δ18.0 (t) and δ46.7 (t) in the ^{13}C-NMR spectrum of **1** were assigned to an lactum carbonyl and three methylene carbons of 2-pyrrolidone moiety. Therefore the structure of **1** was presumed to be *N*-(2-oxopyrrolidinomethyl)cytisine, and determined by comparison with the synthetic sample which was obtained in a 93% yield, by refluxing a mixture of **4**, formalaldehyde and 2-pyrrolidone.

The presence of CONH-CH_2-N< moiety in the molecule of **2** was proposed from the ^{1}H-NMR signals at δ5.73 (1H, broad) due to the amide NH, and at δ4.03 (1H, dd, J =12.2, 5.7Hz) and δ3.84 (1H, dd, J =12.2, 5.7Hz) due to the isolated methylene. The singlet at δ1.98 (3H) was assigned to a methyl group adjacent to a carbonyl group, which was also confirmed by the signal at δ23.4 (q) in the ^{13}C-NMR spectrum. The structure of **2** was presumed to be *N*-(*N*-acetylamino methyl)cytisine, and identified by comparison with the synthetic sample, which was synthesized by refluxing a mixture of **4**, formalaldehyde and *N*-acetamide in EtOH.

The new alkaloids **1** and **2**, which have methylene interposed between two nitrogens (>N-CH_2-N<) in the structures, are regraded as characteristic components in *Maackia hupehensis*, though M. amurensis [unpublished results] and M. floribunda f. pubescens [8] contain (-)-12,12'-methylenedi cytisine which have a methylene group adjacent to the two amino nitrogen.

Alkaloid **3**, colourless needles, mp 217~219 ℃, the molecular formula is $C_{11}H_{13}NO_3$ (*m/z* 207.0882, calc. 207.0894). **3** showed one spot on TLC analysis. However, the ^{1}H-NMR spctrum showed a 3:1 mixture of two components which consideaed to be isomric with each other. The structures of **3** were presumed to be hemiacetals, in which 12-N of **4** is displaced by an oxygen. The major component was presumed to have the ax-OH and the minor one was having eq-OH.

Maackia hupehensis is a plant native to the south of China, and contain a lupinine-type and no sparteine-type. The components of *M. hupehensis* are the same as the south species of Japanese *Maackia*. Further, **1** which contains displaced pyrrolidine ring was also isolated though the corresponded one containing piperidine ring has not been isolated. Biosynthetic pathway of **1** from L-ornithine has been assumed. The relationships of geographical distribution and biosynthesis are very interesting and have being inquired in our laboratories.

EXPERIMENTAL

General procedures. Mps were not corrected. TLC were carried out on silica gel plates in the following solvent systems: 1. CH_2Cl_2-MeOH-25%NH_3H_2O (90 : 9 : 1 or 43 : 6 : 1), 2. CH_2Cl_2-MeOH (4 : 1 or 10 : 1), 3. CH_2Cl_2-AcOEt-MeOH (4 : 4 : 1). The high and low resolution MS were measured at 70 eV using direct inlet system. The ^1H NMR (270 or 500 MHz) and ^{13}C-NMR (125MHz) spectra were recorded using TMS as an internal standard.

Plant material. *Maackia hupehensis* is was collected in May, 1995 and identified by Prof. Jia-shi Li ,Department of pharmacognogy, Beijing University of Traditional Chinese Medicine and Director Ce-ming Tian, Jiang Xi Jiou Jiang Forest and Plant Research. A voucher specimen (No. 74568) is deposited in the Herbarium Institute of Botang Chinese Academy of Sciences Xiangshan. Isolation of alkaloids. The stems of *Maackia hupehensis* were collected in May in Jiang Xi province in the south of China. The air-dried stems (1190g) cut into silces were extracted with 75% MeOH at room temp. The combined extracts were concertrated and acidified with 10% HCl to PH 2. The acid phase was removed by CH_2Cl_2 (x 3) and was basified with 25% NH_3H_2O to PH 11 and extracted with CH_2Cl_2. The basified phase was saturated with K_2CO_3 and extracted with CH_2Cl_2 repeatedly until it became negtive to Dragendorff's reagent. The CH_2Cl_2 extracts were combined and dried on anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The crude alkaloids (5.4g) were obtained as a pale brown oil in a 0.45% yield of the dry stems. The dry leaves (750g) also were treated with the same procedure as described for the stems to give the crude alkaloids (1.3g) in a 0.17% yield.

The crude alkaloid (5.4g) from the stems was subjected to silica gel column (Merck, type 60, 230-400 mesh, 410g) with CH_2Cl_2-MeOH-25%NH_3H_2O (43 : 6 : 1), 250-ml fractions collected, monotoring with TLC, to give 17 frcations. The frcation 1-2 on silica gel column with solvent Et_2O: MeOH : 25%NH_3H_2O (50 : 15 : 1) yielded (-)-*N*-(3-oxobutyl) cytisine (46mg) and (-)-rhombifoline (25mg). From the fraction 6-7 yielded (-)-methylcytisine (0.27g), (-)-*N*-formalycytisine (0.16g). The fractions 8-12 were subjected to silica gel column with solvent CH_2Cl_2-MeOH (4 : 1) yielded cytisine (1.35g) and epibatifoline (1.13g). From the fractions 14-15 on silica gel column with solvent CH_2Cl_2-MeOH-25%NH_3H_2O (50 : 2.5 : 0.5) yielded (-)-lusitanine (0.6g). (+)-epilupinine (0.2g) was separated from the fractions 16-17 with solvent CH_2Cl_2-MeOH (10 : 1).

The basic fraction from leaves was also investigated in the same manner.

Isolation of **1**. The fraction 3 was repeatedly separated by column with solvent system 3 to yield (-)-*N*-(2-oxopyrrolidinomethyl) cytisine (**1**, 9mg), colorless crystals from $CHCl_3$, mp 169-170°C, IR (KBr)/cm^{-1} 1650 (C=O). EI-MS m/z 287.1627 (M^+, calc. for $C_{16}H_{21}N_3O_2$: 287.1625, 13%), 203 (6), 189 (27), 160 (10), 146 (13), 98 (100), 70 (58), 58 (10).

Isolation of **2**. The fraction 5 was separated by column with solvent system CH_2Cl_2-$CH_3COOC_2H_5$-MeOH-25%NH_3H_2O (8 : 8 : 1 : 0.1) to give (-)-*N*-(*N*-acetylaminomethyl) cytisine (**2**, 5mg, oil), IR (KBr)/cm^{-1} 3450 (NH), 1650 (C=O). EI-MS m/z 261.1473 (M^+, calc.

for $C_{14}H_{19}N_3O_2$: 261.1478, 17%), 218 (M^+-CH_3CO, 2), 203 (M^+-CH_3CONH, 24), 190 (85), 189 (60), 160 (23), 147 (100), 146 (76), 58 (93).

Isolation of **3**. The fraction 4 was separated with CH_2Cl_2- AcOEt-MeOH (5 : 5 : 1) to yield (+) hupeol (**3**,8mg).

Synthesis of compound **1** from **4**. (-)-cytisine (**4** , 38mg, 0.2mmol), 35% formalin 0.017ml (0.2mmol) and 2-pyrrolidone 17mg (0.2mmol) were mixed and reflexed in EtOH (2ml) for one hour. The product was chromatography on silica gel column with CH_2Cl_2- AcOEt-MeOH (6 : 6 : 1) to obtain **1** in a yield of 93% (53mg).

Synthesis of compound **2**. It is the same way with synthesis of **1**, reflexing (-)-cytisine (**4**), 35% formalin and *N*-acetamide 0.1mmol, respectively. **2** was separated on silica gel column with solvent system 3 and obtained in a yield of 86% (22mg).

Synthesis of compound **3** from **4**. 12-chlorocytisine, which was derived from (-)-cytisine with NCS, was treated with KOH-EtOH and give 11,12-dehydrocytisine.11,12-dehydrocytisine and $NaNO_2$ were reacted in 5% HCl at 0~5℃ 12 hours to give **3** smoothly in 77% yield..

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(−)−Cytisine からの合成による(+)−hupeol の絶対配置の決定

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【目的】(+)−Hupeol は、中国産*Maackia hupehensis* から見いだされ、(−)−cytisine の様なルピンアルカロイドの基本構造をもつが、12 位窒素が酸素に置換された非アルカロイドであり、極めて珍しい化合物である。(+)−Hupeol が、ルピンアルカロイドの最終代謝産物と考えられている (−)−cytisine から非アルカロイドへの代謝産物であるならば、両化合物の絶対配置は同じはずである。そこで、(+)−hupeol の絶対配置を決定するために、絶対配置既知の (−)−cytisine より合成することを試みた。

【方法・結果】(−)−Cytisine からNCS によるハロゲン化、脱塩酸反応を経て, 2 を得、2 と亜硝酸を−5℃で 24 時間反応させ, (−)−cytisine から 30% の収率で (+)−hupeol {mp 216℃, [α] $^{23}_{D}$ = +33 (EtOH, c=0.4)} を得た。この結果から、(+)−Hupeol の絶対配置 は、(−)−cytisine と同じ (7*R*, 9*R*) と決定され、従って(+)−hupeol は (−)−cytisine の代謝によって生成したものと考えられる。この環状イミンと亜硝酸との反応によるヘミアセタールの生成反応の一般的な応用性については現在検討中です。