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The alkaloids and other constituents from the root and stem of *Aristolochia elegans*

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Abstract—Two new aristolactams, aristolactam E (**1**) and aristolactam-AIIIa-6-*O*-β-D-glucoside (**2**), three novel benzoyl benzyltetrahydroisoquinoline ether *N*-oxide alkaloids, aristoquinoline A (**3**), aristoquinoline B (**4**), and aristoquinoline C (**5**), and a new biphenyl ether, aristogin F (**6**), together with 62 known compounds have been isolated from the root and stem of *Aristolochia elegans* Mast. The structures of the new natural products were established on the basis of spectral evidence. Some of the isolated compounds were examined for their antioxidative and antityrosinase activities. Occurrence of the isoquinolones, biphenyl ethers, and benzoyl benzyltetrahydroisoquinoline ether alkaloids in the same plant indicated the definite possibility of these metabolites as biotransformation intermediates of bisbenzyltetrahydroisoquinoline alkaloids. This can be useful to solve the catabolic process of bisbenzyltetrahydroisoquinoline alkaloids.

1. Introduction

The genus *Aristolochia* (Aristolochiaceae) is found in wide areas, from the tropics to temperate zones and consists of about 400 species. Some species have been used in the form of crude drugs as anodynes, antiphlogistics, antitussives, expectorants, antiasthmatics and detoxicants, especially in China.[1] Five species are native to Taiwan, namely *A. cucurbitifolia*, *A. foveolata*, *A. heterophylla*, *A. kaempferi*, and *A. zollingeriana*. *A. elegans* Mast. (Aristolochiaceae), a woody vine native to Brazil is cultivated as an ornamental plant in Taiwan.[1] It has been reported to contain an alkaloid with uterus contraction stimulating activity.[2] Barnard found that its alcoholic extract showed antimitotic activity.[3] The antiviral activity of this species has also been screened.[4] Previous phytochemical investigations revealed that the leaves, stem, and root of this plant have been a source of numerous lignans, sesquiterpenoids, kaurane diterpenoids, and alkaloids, some of which shown physiological activity.[5–14] As a part of our continuing studies on the *Aristolochia* species, we have reported recently, the isolation of tetralones, isoquinolines, biphenyl ethers, lignans, porphyrins and a diterpene aristolochic acid dimer from the leaves, stem and root of *A. elegans*.[14] The current paper describes the isolation and characterization of two aristolactams, aristolactam E (**1**) and aristolactam-AIIIa-6-O-β-D-glucoside (**2**), three *N*-oxide benzoyl benzyltetrahydroisoquinoline ether alkaloids, aristoquinoline A (**3**), aristoquinoline B (**4**), and aristoquinoline C (**5**), and a biphenyl ether, aristogin F (**6**) from an alkaloid extract of the root and stem of this plant. Some of these isolated compounds were also examined for their antioxidative and anti-tyrosinase activities. The benzoyl benzyltetrahydroisoquinoline alkaloid has been identified for the first time from this plant, which can be considered as an immediate progenitor of bisbenzyltetrahydroisoquinoline alkaloids, important constituents of *A. elegans*.

2. Results and discussion

Aristolactam E (**1**) was obtained as yellow syrup. The molecular formula, $C_{17}H_{13}NO_5$ was established by its HRFABMS ($[M+H]^+$ m/z 312.0875). It presented UV

Keywords: Aristolactams; Benzoyl benzyltetrahydroisoquinoline ether *N*-oxide; Biphenyl ether; Biotransformation intermediates; Bisbenzyltetrahydroisoquinoline alkaloids.
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absorptions at 236, 253, 282, 332, and 390 nm which were reminiscent of a phenanthrene system.[15] The IR absorptions at 3358 (–OH) and 1682 cm^{-1} (lactamic carbonyl) were consistent with typical aristolactam derivative, which was also supported by its positive response with Dragendorff's reagent. The ^1H NMR spectrum also displayed signals for two aromatic methoxyl groups at δ 4.09 and 4.04 (each 3H, s). The two *ortho*-coupled aromatic protons at δ 7.97 and 7.95 (each 1H, d, $J = 8.1$ Hz) were assigned to H-5 and H-6, respectively. The lower field signal for H-5 is diagnostic in aristolactam derivative. The correlations discernible in the ^1H-^1H COSY and NOESY spectra between these two signals proved valuable for further confirmation of this assignment. The two singlets at δ 7.36, and 6.79 each integrating for one proton were attributed to H-2 and H-9, respectively. These data indicated that **1** was 3, 4, 7, 8-tetrasubstituted aristolactam derivative. The NOESY correlations from H-2 to the methoxyl signal at δ 4.04 and from H-6 to the methoxyl singlet at δ 4.05 allowed an unambiguous placement of these methoxyl groups on C-3 and C-7, respectively. On the basis of the above spectral analyses, structure **1** was assigned to aristolactam E (Fig. 1).

Aristolactam-AIIIa-6-O-β-D-glucoside (**2**) was obtained as optically active yellow syrup, showed the pseudomolecular ion peak at m/z 444.1298 in its HRFABMS corresponding to the molecular formula $C_{22}H_{21}NO_9$. A significant fragment ion peak at m/z 282 $[M+H\text{-}162]^+$ in FABMS was indicative of a hexosyl moiety in the molecule. The characteristic UV absorption maxima observed at 236, 253, 282, 332, and 390 nm and IR absorptions at 3400 (–OH) and 1694 cm^{-1} (carbonyl) furthermore suggested the presence of an aristolactam moiety in **2**. The aromatic region of the ^1H NMR spectrum contained an ABX pattern signals at δ 8.94 (1H, d, $J = 2.4$ Hz), 7.79 (1H, d, $J = 8.7$ Hz), and 7.38 (1H, dd, $J = 8.7$, 2.4 Hz) attributable to H-5, H-8, and H-7, respectively. The appearance of H-5 signal in the downfield is due to the deshielding effect of the A-ring in the aristolactam derivatives. Two singlets at δ7.66

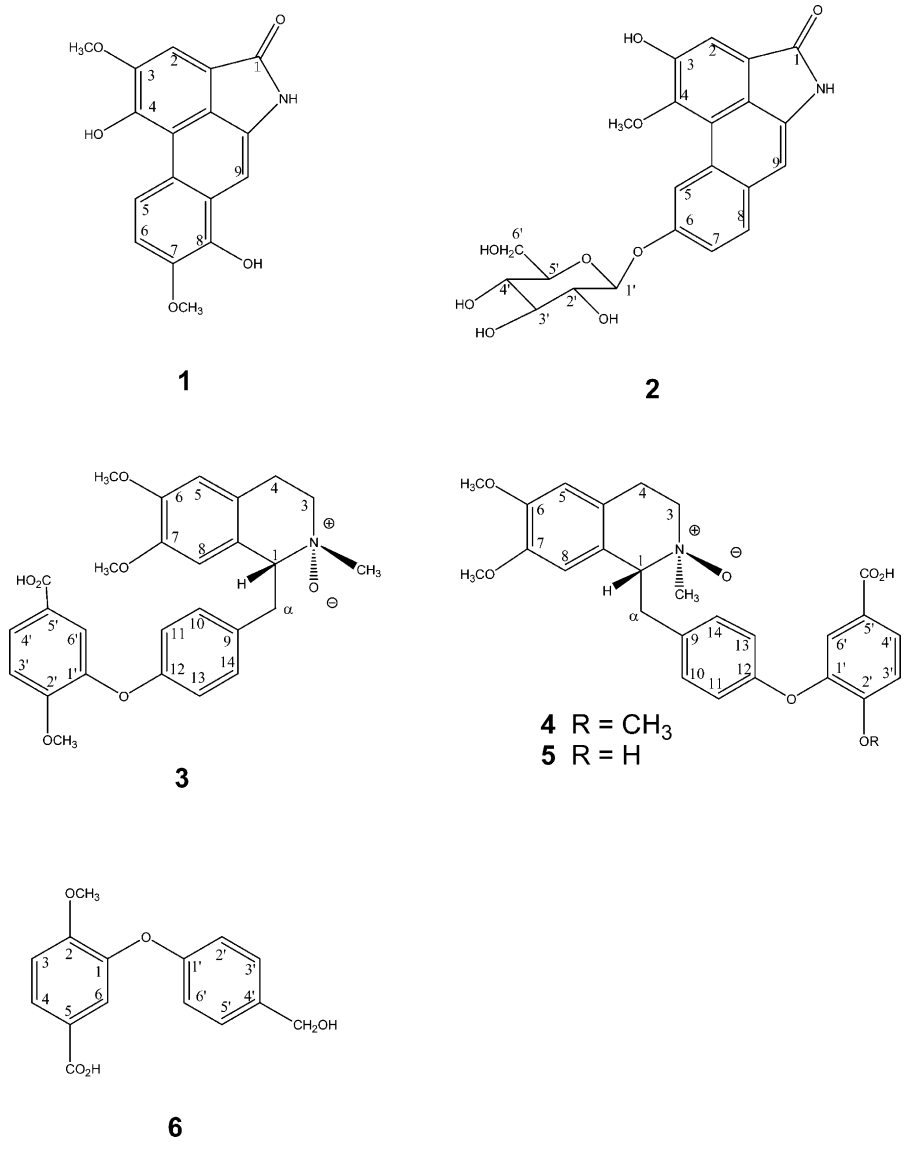


Figure 1. Structures of compounds **1**–**6**.

and 7.06 (each 1H) were assigned to the C-2 and C-9 protons. The ^{1}H NMR spectrum showed the presence of a methxyl group at δ 4.09 (3H, s), and a glucosyl moiety at δ 5.13 (1H, d, J = 6.7 Hz), 3.96 (1H, d, J = 11.3 Hz), 3.81 (1H, dd, J = 11.3, 3.7 Hz), and 3.53 (4H, m). The NOESY experiment showed the NOE correlation between the 4-OMe (δ 4.09) and H-5, and H-1′ (δ 5.13) and H-7, respectively. The combined evidence supported the structure **2** for aristolactam-AIIIa-6-O-β-D-glucoside.

(−)-(*R*)-Aristoquinoline A (**3**) was obtained as optically active syrup. It gave a positive reaction with Dragendorff's reagent, indicating it to be an alkaloid. The strong absorption band in the UV spectrum at 284 nm in conjugation with strong absorption bands in the IR spectrum at 3350, 1712, 1608, and 1415 cm^{-1} suggested that **3** was an oxygenated aromatic alkaloid. The molecular formula of **3** was determined to be $C_{27}H_{29}NO_7$ by HRFABMS, requiring 14 degrees of unsaturation. The ^{1}H NMR spectrum of **3** dissolved in CD_3OD revealed only 28 of the 29 total hydrogens because one hydrogen had exchanged with deuterium from the CD_3OD solvent. The ^{13}C NMR spectrum showed the presence of ten quaternary, ten methine, three methylene, four methyl signals for a total of 27 carbon resonances. The ^{1}H NMR spectrum showed one *N*-methyl at δ 3.19, three *O*-methyl groups at δ 3.82, 3.78 and 3.42, leaving four unassigned oxygens, two of which must be in carboxylic acid functionality from the presence of IR absorption at 1712 cm^{-1} and the carbon signal at δ 174.4 in ^{13}C NMR spectrum. The existence of one oxygen atom as *N*-oxide function was evidenced by the downfield shift of H-α to δ 4.20, *N*-Me to δ 3.19, and the upfield shift of H-1 to δ 4.23. This was also inferred by the base peak at m/z 463 due to the loss of an oxygen atom in the mass spectrum. The remaining one oxygen must be biphenylether. The ^{1}H NMR spectrum contained two aromatic proton singlets at δ 6.48 and 5.66 for H-5 and H-8, aliphatic multiplets at δ 3.85 and 3.46 for H-3 and δ 3.19 (2H) for H-4, and a broad doublet at δ 4.23 for H-1, represents tetrahydroisoquinoline moiety.[16] A *para*-oxygenated benzyl group was supported by HMBC correlations from the two proton aromatic doublets at δ 6.97 (2H, J = 8.5 Hz, H-10, 14) and 6.80 (2H, J = 8.5 Hz, H-11, 13) to an oxygenated aromatic carbon at δ 159.0 (C-12) and from benzylic protons at δ 4.20 (1H, d, J = 11.3 Hz, H-α) and 2.60 (1H, dd, J = 11.3, 11.3 Hz, H-α) to three carbons at δ 132.0 (C-9) and 132.6 (C-10 and C-14). The *para*-oxygenated benzyl moiety was attached to the tetrahydroisoquinoline at C-1 since HMBC correlations were observed from the benzylic protons to the C-1 (δ 79.6) and C-8a (δ 122.0). The aromatic protons of the 1, 2, 5-trisubstituted benzene ring appeared as ABX system at δ 7.82 (1H, dd, J = 8.6, 2.0 Hz, H-4′), 7.61 (1H, d, J = 2.0 Hz, H-6′), and 7.08 (1H, d, J = 8.6 Hz, H-3′). The key NOESY correlation between H-11 and H-6′ established that the ether bridge in **3** was situated between C-12 of benzyl group and C-5′ of trisubstituted benzene ring. The location of the methoxyl substitutions at C-6, 7 and 2′ were determined by NOESY and HMBC experiments. The H-5 aromatic singlet displayed NOESY correlation to OCH_3-6 (δ 3.78), H-8 to OCH_3-7 (δ 3.42), and H-3′ to OCH_3-2′ (δ 3.83). The *N*-methyl singlet at δ 3.19 likewise correlated with the adjacent H-3 resonance as well as with the H-1 in the NOESY spectrum. The HMBC correlations from the methoxyls at δ 3.78, 3.42, and 3.83 to C-6 (δ 150.5), C-7 (δ 148.1) and C-2′ (δ 155.1), respectively, also supported this assignment. Of the 14 degrees of unsaturation indicated by the molecular formula, benzyltetrahydroisoquinoline moiety accounted for nine, while the dioxygenated benzoic acid moiety accounted for another five.

The circular dichroism (CD) curve showed two negative Cotton effects at 246 and 288 nm establishing the absolute stereochemistry at C-1 as *R*,[17] and NOESY crosspeaks between H-1 and H-8 inferred that H-1 was equatorial-orientated. Furthemore, the NOE correlations among *N*-methyl, H-8, and H-4 suggested the absolute configuration of **3** as shown in Figure 2. The α-configuration of the *N*-oxide group was also evidenced from the strong deshielding of the H-α (δ 4.20). The signals for H-8 and the OCH_3-7 were upfield shifted to δ 5.66 and 3.42 indicated the conformation of C-ring was located close to A-ring.[17] Thus the structure **3** was established as (−)-(*R*)-aristoquinoline-A.

(−)-(*R*)-Aristoquinoline B (**4**) was isolated as optically active syrup. It also responded positively with Dragendorff's reagent, suggesting that it was an alkaloid. The molecular formula was established as $C_{27}H_{29}NO_7$ by HRFABMS. From the molecular formula, UV and IR absorptions, and MS spectral data analysis, **4** was determined to be a stereoisomer of **3**. The chemical shifts and coupling patterns in its ^{1}H NMR spectrum were also similar to those of **3**, supported the above proposal. However, the upfield shift of H-α to δ 3.76 inferred the β-configuration for *N*-oxide group.[18] Two negative Cotton effects at 234 and 288 nm in the CD spectrum and the identical NOESY correlations, H-1 (δ 4.46)/H-8 (δ 5.99) and H-1/*N*-methyl (δ 3.35) concluded the same stereochemistry at C-1 as in **3**.[17] In the NOESY spectrum, crosspeaks of H-1 and *N*-methyl with a doublet at δ 7.05 assigned for H-10 and-14 implied the different conformation for C-ring, which is in close proximity of B-ring. It was further evidenced by

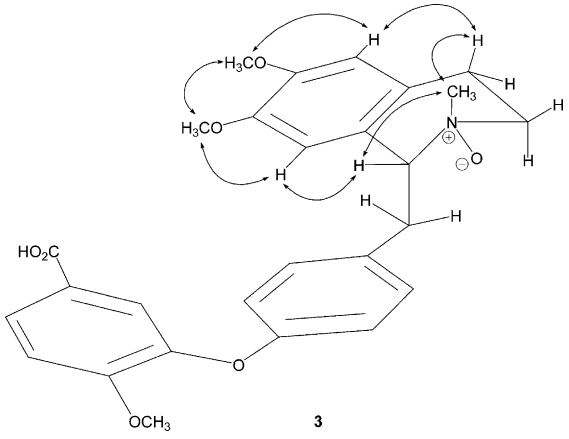
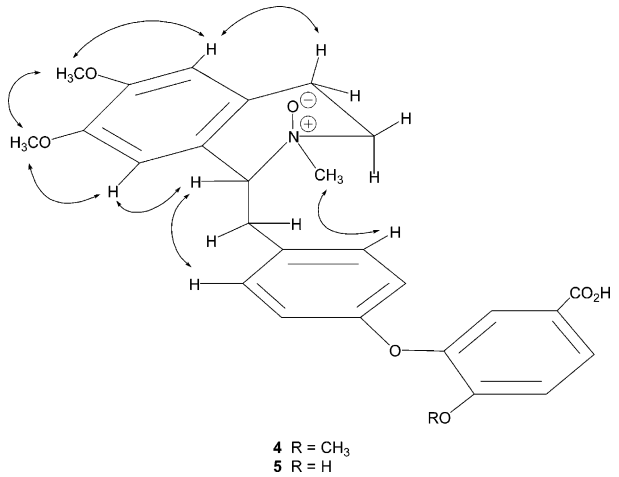


Figure 2. Absolute configuration and key NOESY correlations of compound **3**.

the downfield shifts of H-8 and *N*-methyl group.[17] Thus the structure of (−)-(*R*)-aristoquinoline B was established as **4**.

(−)-(*R*)-Aristoquinoline C (**5**) was obtained as optically active syrup, which also gave a positive reaction with Dragendorff's reagent, indicating it to be an alkaloid. The UV absorption at 274 nm and IR absorption bands at 3353, 1744, 1624, 1585, and 1411 cm^{-1} suggested that **5** was also an oxygenated aromatic alkaloid. The molecular formula of **5**, $C_{26}H_{28}NO_7$, determined from the HRFABMS, was one methylene unit less than that of **4**. The IR data of **5** and the bathochromic shift in the UV spectrum with strong alkali showed the presence of phenolic hydroxyl group. Accordingly, the ^1H NMR spectral data of **5** was very similar to those of **4** except for one less methoxyl signal. An upfield shift of H-3′ signal to δ 6.85 suggested that the hydroxyl group was located on C-2′. The base peak at *m*/*z* 449, due to the loss of an oxygen atom, revealed the presence of an *N*-oxide function, whose β-orientation was deduced from the downfield shift of H-1 (δ 4.46) and *N*-Me (δ 3.35). The absolute configuration (1*R*) for **5** was assigned directly from the two negative Cotton effects at 233 and 288 nm in the CD spectrum.[17] In the NOESY spectrum, it also exhibited correlations among H-1 (δ 4.46), H-10 (δ 7.05), and *N*-methyl (δ 3.35), which also established the conformation of C-ring lying in the proximity of B-ring. In conclusion, the absolute configuration of **5** was deduced as shown in figure 3. Therefore, the structure **5** was established as (−)-(*R*)-aristoquinoline C.

Aristogin F (**6**) was isolated as colorless amorphous powder. The ^1H NMR signals of **6** at δ 7.89 (1H, dd, J = 8.4, 2.0 Hz, H-4), 7.64 (1H, d, J = 2.0 Hz, H-6), and 7.04 (1H, d, J = 8.4 Hz, H-3); 7.33 (2H, d, J = 8.4 Hz, H-3′, 5′) and 6.96 (2H, d, J = 8.4 Hz, H-2′, 6′), were virtually identical to those observed for 2,5,4′-trisubstituted biphenyl ether backbone in aristogin-E (**16**).[19] A strong carbonyl band in the IR spectrum at 1720 cm^{-1} combined with the three proton singlet at δ 3.92 and two proton singlet at δ 4.67 in the ^1H NMR spectrum inferred the three substituents as a carboxylic acid, a methoxyl, and a hydroxymethyl group. The NOE connectivities from oxymethylene protons (δ 4.67) to H-3′ and 5′ (δ 6.96) as well as from methoxyl protons (δ 3.92) to H-3 (δ 7.04) clear established the placement of these groups on C-4′ and C-2′, respectively. Consequently, the carboxylic acid group was attached to C-5 which was further supported by the downfield shift of H-4 and H-6. The aforesaid spectral discussion suggested the structure **6** as aristogin-F.



4 R = CH_3
5 R = H

Figure 3. Absolute configuration and key NOESY correlations of compound **4** and **5**.

The known compounds, five aristolactams, 9-methoxyaristolactam I (**7**),[20] aristolactam AII (**8**),[21] isoaristolactam AII (**9**),[22] aristolactam AIIIa (**10**),[23] and aristolactam C *N*-β-D-glucoside (**11**);[24] seven biphenyl ethers, aristogin-A (**12**),[19]-B (**13**),[19]-C (**14**),[14]-D (**15**),[19]-E (**16**)[19] and 4-methoxy-3, 4′-oxydibenzoic acid (**17**);[25] four tetralones, aristelegone-A (**18**),[19]-B (**19**),[19]-C (**20**),[19] and [19]-D (**21**); five isoquinolone, pericampylinone-A (**22**),[19] corydaldine (**23**),[14] thalifoline (**24**),[14] northalifoline (**25**),[14] and *N*-methylcorydaldine (**26**);[14] ten lignans, aristelegin-A (**27**),[19]-B (**28**),[19]-C (**29**),[19] (−)-cubebin (**30**),[26] α-methylcubebin (**31**),[27] β-methylcubebin (**32**),[27] (−)-hinokinin (**33**),[28] (−)-5″-methoxyhinokinin (**34**),[29] (−)-kobusin (**35**),[30] and (+)-medioresinol (**36**);[31] one dimer, aristolin (**37**);[19] four diterpenoids, *ent*-kauran-16β,17-diol (**38**),[32] *ent*-16β,17-epoxykauran (**39**),[32] *ent*-kaur-15-en-17-ol (**40**),[33] and *ent*-15β,16-epoxykauran-17-ol (**41**);[33] one sesquiterpenoid, aristololide (**42**);[13] four amides, *N-trans*-feruloyltyramine (**43**),[34] *N-cis*-feruloyltyramine (**44**),[34] *N-trans*-cinnamyltyramine (**45**),[35] and *N-p-trans*-coumaroyltyramine (**46**);[36] three aristolochic acids, aristolochic acid D (**47**),[37] aristoloside (**48**),[38] and aristolochic acid I (**49**);[39] four aristolochic acid methyl esters, aristolochic acid IV methyl ester (**50**), [38] methylaristolochate (**51**),[40] aristolochic acid D methyl ester (**52**),[38] and aristolochic acid-Ia methyl ester (**53**),[40] four aporphines, magnofoline (**54**),[41] oxonuciforine (**55**),[41] isomoschatoline (**56**),[42] and 4,5-dioxodehydroasimilobine (**57**);[43] eight benzenoids, methylparaben (**58**),[44] methyl vanillate (**59**),[45] *p*-hydroxybenzaldehyde (**60**),[46] vanillin (**61**),[47] methyl 4-hydroxy-3-methoxycinnamate (**62**),[48] cinnamic acid (**63**),[49] ω-hydroxypropioguaiacone (**64**),[50] and ficusol (**65**);[51] two steroids, β-sitosterol (**66**) [47] and β-sitosteryl glucoside (**67**);[52] and phellochrysein (**68**)[53] were also isolated and identified by comparison of their spectroscopic data with literature values.

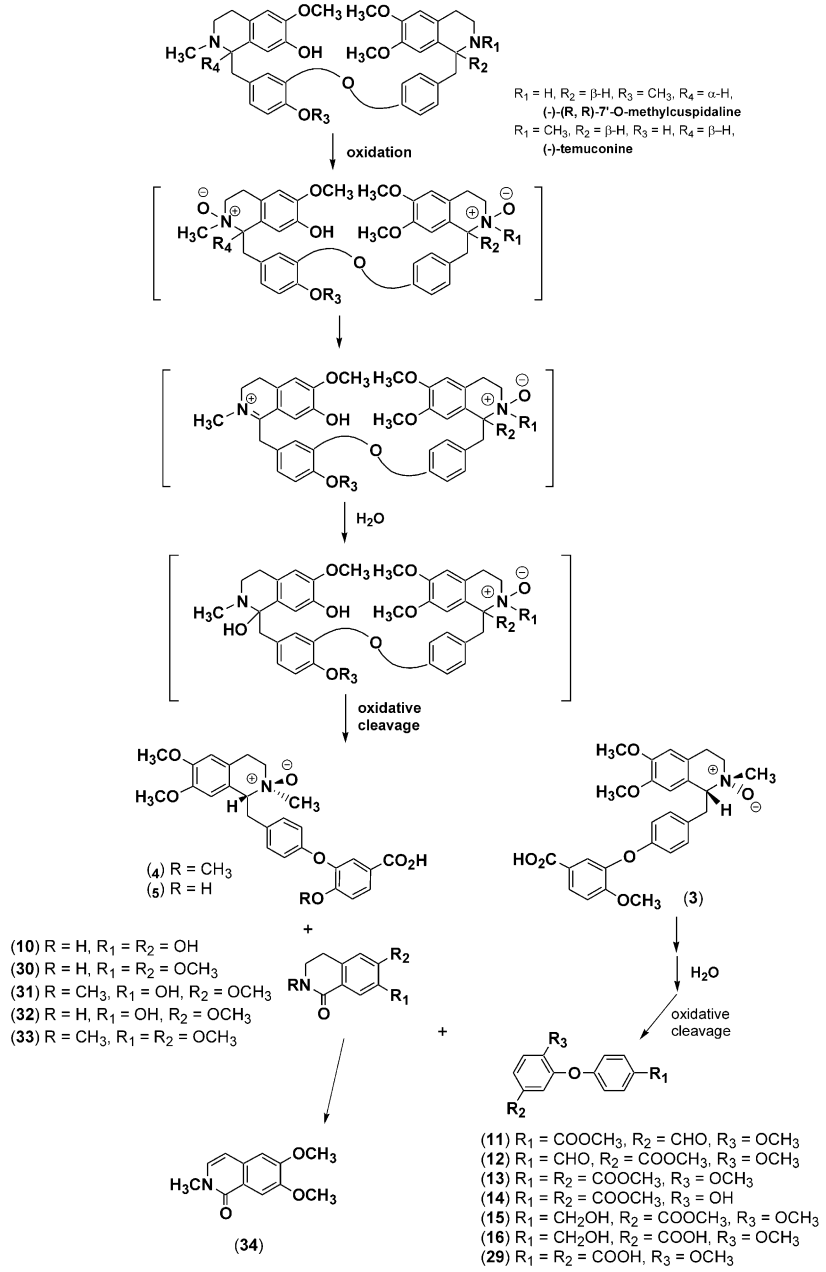
Compound **24**, **30**, **38**, **39** and **40** were examined for their antioxidative properties using the α,α-diphenyl-β-picrylhydrazyl free radical (DPPH) assay by the reported method.[54, 55] Compound **24**, **30**, **38**, and **39** were found to be inactive at 50 μM with inhibition percentages 21.5%, 15.3%, 10.3%, and 10.4%, respectively, compared to the reference compound vitamin E (IC_{50}, 8.3 μM). In addition, the antityrosinase activities were also evaluated using the reported procedures.[55] Compound **24**, **30** and **38** were inactive as tyrosinase inhibitors at 50 μM, with 6.0%, 22.9%, and 27.7% inhibition, compared to the reference compound kojic acid (IC_{50} value 125 μM).

Within the intricate mosaic of isoquinoline alkaloids, it can be stated that the dimerization of two enantiomeric

benzylisoquinoline units can lead to a bisbenzylisoquinoline through phenolic oxidative pathway. In contrast, oxidative cleavage of bisbenzyltetrahydroisoquinolines through *N*-oxide to produce tetrahydroisoquinolone-benzyltetrahydroisoquinoline dimers as well as that of a simple monomeric benzyltetrahydroisoquinoline to tetrahydroisoquinolone and biphenyl ether is an intrinsic part of the general alkaloid catabolic process. It has also been adumbrated that the tetrahydroisoquinolone alkaloids originate in plants from the oxidation of simple benzyltetrahydroisoquinolines. Some bisbenzyltetrahydroisoquinolines, such as (−)-(*R*, *R*)-methylcuspidaline[51] and (−)-temuconine,[53] were found in Brazilian species of *A. elegans*. Since we have obtained tetrahydroisoquinolones **22**–**26**, biphenyl ethers **6**, **7**, **13**–**17**, and *N*-oxide benzoyl benzyltetrahydroisoquinoline ethers **3**–**5** from *A. elegans*, and the striking structural kinship of these metabolites with the bisbenzyltetrahydroisoquinolines, it was considered as a definite possibility that these metabolites were derived biogenetically from bisbenzyltetrahydroisoquinolines in general alkaloid catabolic process. The possible biogenetic transformation pathway was represented in Scheme 1 for the first time on the basis of evidences from natural sources.[56,57] The proposed biogenetic transformation pathway can be helpful to solve the catabolic process of structurally interesting bisbenzyltetrahydroisoquinolines.

3. Conclusion

Six new and 62 known compounds, were isolated from the root and stem of *A. elegans*. The occurrence of



Scheme 1. Possible biotransformation pathway from bisbenzyltetrahydroisoquinolines.

aristoquinoline A, B, and C constitute the first report of *N*-oxide benzoyl benzyltetrahydroisoquinoline ether alkaloids from *Aristolochia* species. Our results provided the natural evidence for the catabolic process of structurally interesting bisbenzyltetrahydroisoquinolines. From this study, we concluded that the isoquinolones, benzylisoquinolines, biphenyl ethers, and *N*-oxide benzoyl benzyltetrahydroisoquinoline ether alkaloids were derived biogenetically from bisbenzylisoquinolines, common metabolites of *Aristolochia* species, in general alkaloid catabolic process. Thus, we have outlined the possible biotransformation pathway of bisbenzylisoquinolines from our results for the first time based on the evidences from the natural source, which can be helpful to solve the catabolic process of bisbenzyltetrahydroisoquinolines.

4. Experimental

Melting points were determined on a Yanaco MP-S3 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi UV-3210 spectrophotometer. IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-200, Avance-300, AMX-400 and Varian-400 Unity Plus spectrometers using tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out with silica gel [Kieselgel 60, 70–230 mesh (Aldrich)]. TLC was conducted on 0.25 mm precoated silica gel plates (60F254, Merck). EI and HR-EI mass spectra were measured on a VG-70-250S spectrometer by a direct inlet system. FAB and HR-FAB mass spectra were obtained on a Jeol JMS-700 spectrometer. CD spectra were recorded with a Jasco J-720 spectropolarimeter.

4.1. Plant material

Aristolochia elegans Mast. was collected in May 1992, from Tainan Hsien, Taiwan, and authenticated by Prof. C. S. Kuoh. A voucher specimen of the plant (NCKU Wu 92008) has been deposited at the herbarium of National Cheng Kung University, Tainan, Taiwan.

5. Extraction and isolation

Fresh root and stem of *A. elegans* Mast. (3.1 kg) were extracted with hot MeOH (20 L × 9) and concentrated to give a dark brown syrup which was partitioned between H_2O and $CHCl_3$, and then *n*-BuOH. This resulted in $CHCl_3$, *n*-BuOH, H_2O and insoluble portions after evaporating the solvent. The $CHCl_3$ solubles were chromatographed over silica gel using a gradient of C_6H_6 and Me_2CO to afford 5 fractions. Fraction 1 was rechromatographed over silica gel using mixture of C_6H_6 and Me_2CO as eluents, and purified by preparative TLC to yield **8** (2 mg), **9** (1 mg), **10** (3 mg), **18** (24 mg), **19** (27 mg), **27** (4 mg), **37** (4 mg), **39** (451 mg), **40** (312 mg), **41** (3 mg), **42** (8 mg), **30** (2 mg), **33** (37 mg), **334** (10 mg), **35** (1 mg), **50** (1 mg), **58** (5 mg), **59** (2 mg), **62** (3 mg), and **68** (1 mg). Fraction 2 on chromatography yielded **3** (1 mg), **6** (2 mg), **20** (2 mg), **21** (2 mg), **28** (7 mg), **38** (393 mg), **26** (12 mg), **36** (11 mg), **45** (1 mg), **64** (2 mg), **65** (3 mg), and **66** (343 mg). Fraction 3 gave **24** (12 mg) and **5** (10 mg). Fraction 4 resulted **43** (7 mg), **44** (6 mg), and **63** (4 mg). Fraction 5 gave **11** (3 mg), **12** (2 mg), **29** (2 mg), **23** (8 mg), **55** (7 mg), **56** (1 mg), **60** (1 mg), **61** (1 mg), and **67** (33 mg). The *n*-BuOH portion and insoluble portion was chromatographed over cation exchange resin eluting with water to give a fraction containing non-alkaloids, followed by eluting with 5% NH_3 solution to give a fraction containing alkaloids. The non-alkaloid fraction was rechromatographed over Sephadex LH-20 using a gradient of H_2O and CH_3OH to afford **7** (3 mg), **17** (2 mg), **31** (7 mg), **32** (3 mg), **46** (2 mg), **47** (3 mg), **48** (5 mg), **51** (1 mg), **52** (2 mg) and **57** (3 mg). Finally, the combined alkaloid fraction of *n*-BuOH and insoluble portions were chromatographed over a C-18 column eluting with a gradient of H_2O and CH_3OH to produce **1** (2 mg), **2** (3 mg), **4** (1 mg), **7** (3 mg), **12** (2 mg), **13** (4 mg), **14** (2 mg), **15** (3 mg), **16** (1 mg), **18** (2 mg), **22** (7 mg), **23** (10 mg), **24** (8 mg), **25** (5 mg), **26** (6 mg), **29** (1 mg), **47** (2 mg), **49** (2 mg), **53** (4 mg), **54** (10 mg), and **62** (12 mg).

5.1. Aristolactam E (1)

Yellow syrup; UV (MeOH) λ_{max} (log ε) 236 (4.22), 253 (4.12), 282 (3.65), 332 (3.52), 390 (3.44) nm; IR (KBr pellet) ν_{max} 3358, 1682, 1432, 1280 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), δ 4.04 (s, 3H, OCH_3), 4.09 (s, 3H, OCH_3), 6.79 (s, 1H, H-9), 7.36 (s, 1H, H-2), 7.95 (d, 1H, J = 8.1 Hz, H-6), 7.97 (d, 1H, J = 8.1 Hz, H-5); FABMS m/z 312 ([M + H]$^+$, 1); HRFABMS m/z 312.0875(MH$^+$ calcd for $C_{17}H_{14}NO_5$, 312.0872).

5.2. Aristolactam F (2)

Yellow syrup, $[\alpha]_D^{25}$ −7.8° (c 0.7, MeOH). (MeOH) UV λ_{max} (log ε) 240 (4.55), 246 (4.33), 280 (4.23), 290 (4.15), 324 (4.02) nm; IR (KBr pellet) ν_{max} 3400, 1692, 1632, 1422, 1365 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), δ 3.53 (m, 4H, H-2′, 3′, 4′, 5′), 3.81 (dd, 1H, J = 11.3, 3.7 Hz, H-6′), 3.96 (d, 1H, J = 11.3 Hz, H-6′), 4.09 (s, 3H, OCH_3), 5.13 (d, 1H, J = 6.7 Hz, H-1′), 7.06 (s, 1H, H-9), 7.38 (dd, 1H, J = 8.7, 2.4 Hz, H-7), 7.66 (s, 1H, H-2), 7.79 (d, 1H, J = 8.7 Hz, H-8), 8.94 (d, 1H, J = 2.4 Hz, H-5); FABMS m/z 444 ([M + H]$^+$, 2), 264 (3); HRFABMS m/z 444.1298 (MH$^+$ calcd for $C_{22}H_{22}NO_9$, 444.1295).

5.3. (−)-(*R*)-Aristoquinoline A (3)

Yellowish syrup; $[\alpha]_D^{25}$ −31.1 (c 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 234 (4.26), 284 (3.75) nm; IR (KBr pellet) ν_{max} 3350, 1712, 1608, 1415 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), δ 2.60 (dd, 1H, J = 11.3, 11.3 Hz, H-α), 3.19 (s, 3H, NCH_3), 3.19 (m, 2H, H-4), 3.42 (s, 3H, OCH_3-7), 3.46 (m, 1H, H-3), 3.78 (s, 3H, OCH_3-6), 3.82 (s, 3H, OCH_3-2′), 3.85 (m, 1H, H-3), 4.20 (br. d, 1H, J = 11.3 Hz, H-α), 4.23 (br. d, 1H, J = 11.3 Hz, H-1), 5.66 (s, 1H, H-8), 6.78 (s, 1H, H-5), 6.80 (d, 2H, J = 8.5 Hz, H-11, 13), 6.97 (d, 2H, J = 8.5 Hz, H-10, 14),

7.08 (d, 1H, $J=8.6$ Hz, H-3′), 7.61 (d, 1H, $J=2.0$ Hz, H-6′), 7.82 (dd, 1H, $J=8.6$, 2.0 Hz, H-4′); ^{13}C NMR (75 MHz, CD_3OD), δ 25.3 (t, C-4), 38.7 (t, C-α), 56.1 (q, NCH_3), 56.4 (q, 3×OCH_3), 60.5 (t, C-3), 79.6 (d, C-1), 112.6 (d, C-8, 3′), 112.9 (d, C-5), 117.4 (d, C-11, 13), 122.0 (s, C-8a), 124.2 (d, C-6′), 126.4 (s, C-4a), 128.1 (d, C-4′), 132.0 (s, C-9), 132.6 (d, C-10, 14), 144.7 (s, C-1′), 148.1 (s, C-7), 150.5 (s, C-6), 155.1 (s, C-2′), 159.0 (s, C-12), 174.4 (s, COOH); FABMS m/z 480 ($[M+H]^+$,7), 463 (8), 437 (18); HRFABMS m/z 480.2024 (MH^+ calcd for $C_{27}H_{30}NO_7$, 480.2022); CD (MeOH, c 6.57×10^{-4}): $[\theta]_{228}$ 5.50×10^2, $[\theta]_{246}$ -7.72×10^3, $[\theta]_{265}$ -1.18×10^3, $[\theta]_{288}$ -4.48×10^3, $[\theta]_{300}$ 1.68×10^2.

5.4. (−)-(*R*)-Aristoquinoline B (4)

Yellowish syrup; $[\alpha]_D^{25}$ −77.1° (c 0.016, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.20), 280 (3.75) nm; IR (KBr pellet) ν_{max} 3350, 1712, 1623, 1461 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), δ 2.86 (dd, 1H, $J=10.8$, 8.1 Hz, H-α), 2.97 (m, 1H, H-4), 3. 31 (m, 1H, H-4), 3.35 (s, 3H, NCH_3), 3.47 (s, 3H, OCH_3-7), 3.47 (m, 1H, H-3), 3.76 (s, 3H, OCH_3-6), 3.76 (m, 1H, H-α), 3.79 (s, 3H, OCH_3-2′), 3.81 (m, 1H, H-3), 4.46 (d, 1H, $J=8.1$ Hz, H-1), 5.99 (s, 1H, H-8), 6.76 (s, 1H, H-5), 6.80 (d, 2H, $J=8.3$ Hz, H-11, 13), 7.05 (d, 2H, $J=8.3$ Hz, H-10, 14), 7.08 (d, 1H, $J=8.4$ Hz, H-3′), 7.62 (d, 1H, $J=1.8$ Hz, H-6′), 7.83 (dd, 1H, $J=8.4$, 1.8 Hz, H-4′); FABMS m/z 480 ($[M+H]^+$, 7), 463 (5); HRFABMS m/z 480.2026 (MH^+ calcd for $C_{27}H_{30}NO_7$, 480.2022); CD (MeOH, c 3.56×10^{-4}): $[\theta]_{234}$ -3.87×10^3, $[\theta]_{266}$ -2.22×10^3, $[\theta]_{288}$ -8.13×10^3.

5.5. (−)-(*R*)-Aristoquinoline C (5)

Yellowish syrup; $[\alpha]_D^{25}$ −52.0 (c 0.014, MeOH); UV(MeOH) λ_{max} (log ε): 248 (4.24), 274 (3.85) nm; IR (KBr pellet): ν_{max} 3353, 1711, 1624, 1585, 1411, 1277 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), δ 2.86 (dd, 1H, $J=13.1$, 7.2 Hz, H-α), 2.98 (m,1H, H-4), 3.31 (m, 1H, H-4), 3.35 (s, 3H, NCH_3), 3.45 (m, 1H, H-3), 3.47 (s, 3H, OCH_3-7), 3.80 (dd, 1H, $J=13.1$, 4.8 Hz, H-α), 3.83 (m, 1H, H-3), 3.83 (s, 3H, OCH_3-6), 4.46 (d, 1H, $J=7.2$ Hz, H-1), 6.03 (s, 1H, H-8), 6.76 (s, 1H, H-5), 6.85 (d, 1H, $J=8.4$ Hz, H-3′), 6.88 (d, 2H, $J=8.5$ Hz, H-11, 13), 7.05 (d, 2H, $J=8.5$ Hz, H-10, 14), 7.56 (d, 1H, $J=2.1$ Hz, H-6′), 7.67 (dd, 1H, $J=8.4$, 2.1 Hz, H-4′). FABMS m/z 466 ($[M+H]^+$, 1), 450 (1); HRFABMS m/z 466.1865 (MH^+ calcd for $C_{26}H_{28}NO_7$, 466.1864); CD (MeOH, c 3.14×10^{-4}): $[\theta]_{218}$ -1.17×10^4, $[\theta]_{224}$ -7.07×10^3, $[\theta]_{233}$ -1.22×10^4, $[\theta]_{269}$ -7.70×10^1, $[\theta]_{288}$ -1.78×10^3.

5.6. Aristogin F (6)

Colorless powder, UV (MeOH) λ_{max} (log ε) 248 (4.34), 284 (3.83, sh) nm; IR (KBr pellet) ν_{max} 3350, 1720, 1608, 1455 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), δ 3.92 (s, 3H, OCH_3), 4.67 (s, 2H, CH_2), 6.96 (d, 2H, $J=8.4$ Hz, H-2′, 6′), 7.04 (d, 1H, $J=8.4$ Hz, H-3), 7.33 (d, 2H, $J=8.4$ Hz, H-3′, 5′), 7.64 (d, 1H, $J=2.0$ Hz, H-6), 7.89 (dd, 1H, $J=8.4$, 2.0 Hz, H-4); EIMS m/z 274 (M^+, 21), 328 (83), 300 (21), 180 (21), 116 (100); HREIMS m/z 274.0842 (M^+ calcd for $C_{15}H_{14}O_5$, 274.0841).

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