



REVIEW

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Review on NMR spectroscopic data and recent analytical methods of aristolochic acids and derivatives in *Aristolochia* herbs

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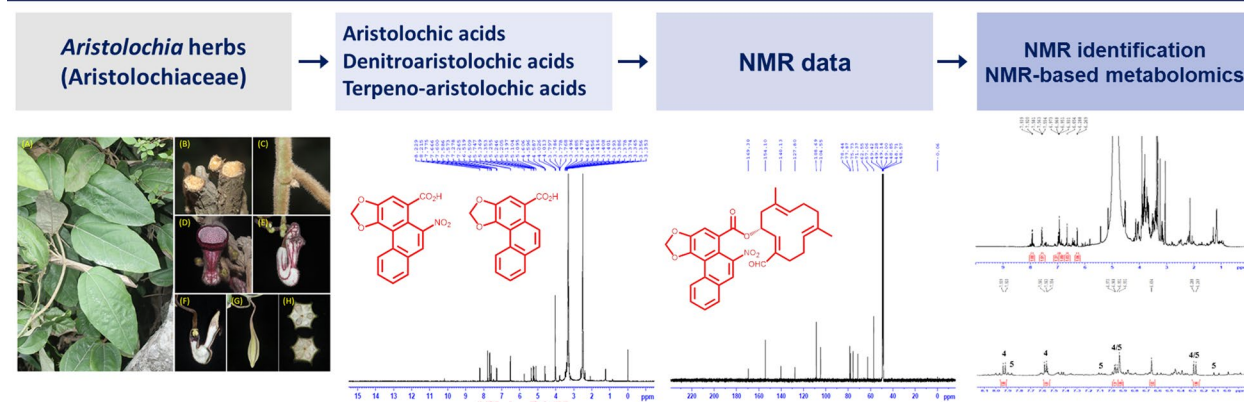
Abstract

Aristolochic acids (AAs) are an important group of secondary metabolites in the genus *Aristolochia*. The presence of aristolochic acids infers the potency of many *Aristolochia* herbs used for ages in traditional medicine of China, Europe, Central America, India, and some other countries. Although being moderately cytotoxic, intake of AAs is associated with serious health problems, such as nephrotoxicity and carcinogenicity. Analyzing AAs in *Aristolochia* herbs is crucial for regulating their efficacy and toxicity because phytochemistry works have shown the occurrence of AAs in almost all *Aristolochia* herbs studied. Using two-dimensional parameters, chemical shifts and coupling constants, NMR spectroscopy is a modern, accurate, and reliable method in the analysis of secondary metabolites. Comparing experimental spectroscopic data with those of known and related compounds helps simplify the structural identification of secondary metabolites. The compilation of an NMR database of AAs from scattered sources would also be useful in NMR-based metabolomics. The present review provides updated information on sources and NMR spectroscopic data of 54 aristolochic acid derivatives, including AAs and their methyl esters, denitroaristolochic acids and their derivatives, and sesqui- and diterpene esters of AAs. The report also covers the newest development of analytical and preparative methods used in separation, identification, and quantification of AAs in *Aristolochia* herbal samples.

Keywords *Aristolochia*, Aristolochiaceae, Aristolochic acid (AA), Denitroaristolochic acid, LC analysis, NMR data

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Graphical Abstract

NMR of aristolochic acids in *Aristolochia* herbs

1 Introduction

The genus *Aristolochia* comprises more than 500 plant species that grow in wide areas, from tropics to temperate zones. For a long time, members of the genus have been in records for medicinal use in Europe, Asia, Africa, and Central America [1, 2]. Certain *Aristolochia* species have a long tradition to be used in China as popular medicaments in obstetrics, in the treatment of festering wounds, asthma, inflammation, and tumors, and as anodynes, expectorants, and tonics [1, 3–11]. A thorough review published in 2004 lists traditional/folklore medicinal uses of 35 *Aristolochia* species [12]. More than sixty species of *Aristolochia* species have been the subjects of phytochemical and pharmacological studies over the past 70 years [12]. Various types of compounds have been reported from the isolation works, and aristolochic acids (AAs), aristolactams, alkaloids (aporphines, tetrahydroisoquinolines, benzyloisoquinolines, and bisbenzyloisoquinolines), terpenoids, lignoids, flavonoids (flavones, dihydroflavonols, isoflavonols, biflavones, chalcone-flavones, and tetraflavonoids), coumarins, and quinones are the most common types of compounds [12, 13].

Aristolochic acids are naturally occurring nitrophenanthrenic compounds in *Aristolochia* species of the family Aristolochiaceae, with aristolochic acids I and II usually being the most abundant. In 2014, the chemistry, biosynthesis, and pharmacology of AAs were reviewed [14]. The only source of these substances is the plant itself, and there is currently no large-scale method to efficiently synthesize AAs [15]. The interest in AAs is linked with their possible development into immunostimulants and

anticancer agents [16]. Aristolochic acids have been experimentally and clinically demonstrated to be one of the most bioactive constituents of *Aristolochia* herbs, however, the toxicity of these compounds must be understood. Aristolochic acids I and II are the main risk factors for nephropathy and mutagenicity during chronic use of *Aristolochia* herbs for the treatment of rheumatism, diuretics, and analgesics [17]. Intracellular depletion of GSH by aristolochic acid I and long metabolism of aristolochic acids into active intermediates during the detoxification process are the postulated mechanisms for the toxicity [14, 18–20]. In vivo, AAs contribute to the formation of adducts with DNA that result in DNA mutations, leading to the promotion of cancer [14, 20, 21]. In addition, they are the precursors to be metabolized to toxic aristolactams, which may be responsible for the secondary toxicity of *Aristolochia* herbs. Aristolochic acids are also the hypothetical precursors of naturally occurring tariacuripyrones (5-nitro-benzo[h]chromen-2-ones) from *A. brevipes* [22, 23] and aristchamics A and B from *A. championii* [24], whose biological activities and toxicity are not fully understood. Despite being warned on the risks associated with herbal products containing aristolochic acids by reputable official organizations such as the FDA (USA Food and Drug Administration), the WHO International Agency for Research on Cancer, and the Medicines and Healthcare Products Regulatory Agency (MHRA), many websites continue to recommend *Aristolochia* herbal products [25]. Before new regulations to control the safety of these health botanical products may be implemented, more research is needed to ascertain the

presence and amounts of AAs in *Aristolochia* plants and to investigate the structure–activity/toxicity relationship between structures of AAs and their curative as well as toxic effects. Several studies pointed out the nitro group as a structural requirement for cytotoxicity of AAs, and the presence or absence of methoxy or hydroxyl groups may mediate cytotoxicity [14, 26]. It is consistent with the emphasized role of the nitro group in metabolic conversion into harmful intermediates. Moreover, the toxicity rises with the increased number of methoxy groups [14].

Most of the reviews on NMR data of AAs were published prior to 1990. In 1982, 1984, and 1989, the ^1H -NMR spectroscopic data of AA Ia, AA I (AA A, aristolochic acid), and AA D (AA IVa) [27] and ^{13}C -NMR spectroscopic data of some natural (AA I, AA Ia, AA II, AA III, AA IIIa, AA IV, AA IVa, AA Va, aristolic acid and its methyl ester) and synthetic phenanthrene derivatives have been reviewed [27–29]. The ^{13}C -NMR data of *ent*-kauranyl aristolochates, aristolin, aristolin I from *A. elegans*, and aristolin II from *A. pubescens* were reported [13]. Recently, more AA derivatives have been isolated and assigned with ^1H - and ^{13}C -NMR techniques, but their spectroscopic data are scattered in the literature. The application of NMR is still valid in the identification of AAs in *Aristolochia* and other plant species, either by isolation or by using NMR-based metabolomics techniques [30]. The aim of the present review is to compile an up-to-date list of NMR spectroscopic data of AAs and their derivatives. In addition, published LC and NMR analytical approaches in the analysis of AAs have been reviewed from the current literature.

2 Occurrence of aristolochic acids and their derivatives

According to their structural features, compounds discussed in this review form three characteristic groups: AA derivatives, denitroaristolochic acid derivatives, and sesqui- and diterpene esters of AAs.

2.1 Aristolochic acids, their sodium salts, and their methyl esters

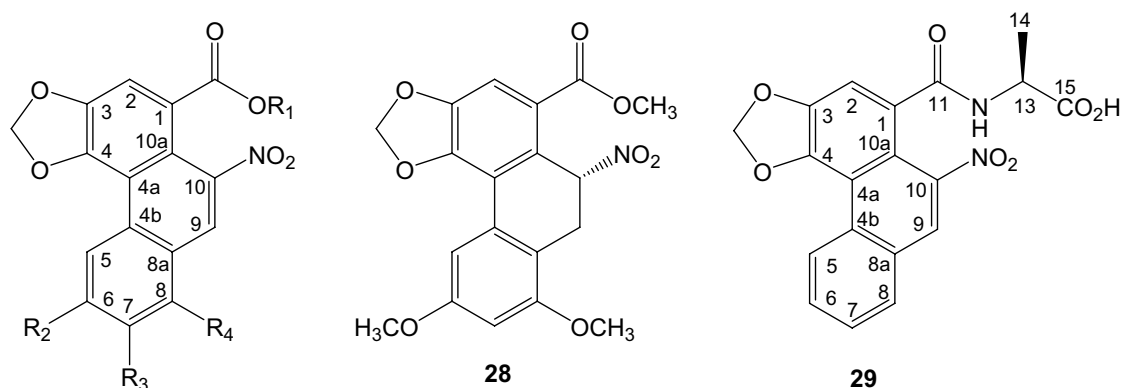
Aristolochic acid derivatives isolated from *Aristolochia* species are listed in chronological order (Fig. 1). Aristoloslone (1) was isolated from the stems of *A. manshuriensis* [31] and its semisynthetic methyl ester (2), AA III, AA IV (3), and AA III methyl ester (4) from the root of *A. longa* [32] and from the root and stems of *A. cucurbitifolia* [33]; AA IV methyl ester (5) was synthesized from 3 using diazomethane in excess [32], methyl aristolochate (6) from the root of *A. indica* [34], 3-hydroxy-4-methoxy-10-nitrophenanthrene-1-carboxylic acid methyl ester (methyl ester of 3-hydroxy-4-methoxy equivalent of AA

II) (7) from the stems of *A. liukuensis* (*syn. A. kaempferi*) [35] and the root of *A. auricularia* [16], ariskanins A–E (8–12) from the root and stems of *A. kankauensis* [36], AA IIIa 6-*O*- β -D-glucoside (13) from the root of *A. cinabarinata* [37], sodium aristolochate-I (14), sodium aristolochate-C (sodium aristolochate IIIa) (15), and sodium 7-hydroxyaristolochate A (16) from the leaves of *A. foveolata* [38], sodium aristolochate I (14), sodium aristolochate II (17), sodium aristolochate IIIa (15), sodium aristolochate IVa (18), and AA VIIa (19) from the tubercula of *A. pubescens* [39], aristolochic acid-VII methyl ester (20) from the fresh leaves of *A. cucurbitifolia* [40], sodium aristolochate (14) and sodium aristolochate-VII (21) from the root and stems of *A. heterophylla* [41], AA-Ia methyl ester (22) from the root and stems of *A. kaempferi* [42], AA-D (AA IVa) (23) from the leaves and stems of *A. bracteolata* [43], AAs B, C, F, and G (24–27) from the root of *A. fangchi* [17], aristochamic A (28) from the rhizomes of *A. championii* [24], AA II (24) and AA VIIa (19) from the roots of *A. contorta* [18], and AA II alanine amide (29) from the whole plant of *A. maurorum* [44].

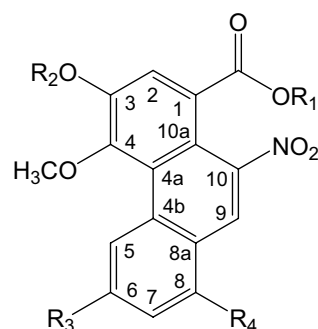
The substitution of aromatic carbons from C-5 to C-8 of the C-ring of the phenanthrene nucleus may greatly influence the cytotoxicity of AAs. The most cytotoxic is aristolochic acid I with an 8-OMe substituent, followed by aristolochic acid II (unsubstituted), whereas aristolochic acid III (6-OMe) is nontoxic [19]. The cytotoxic potency of AA I is significantly reduced when the 9-hydroxy group is introduced [26]. A reducing trend of cytotoxicity is also observed with 6-hydroxy, 7-hydroxy, and 6,8-dihydroxy substitution [17, 18].

2.2 Denitroaristolochic acids, their sodium salts, and their methyl esters

Aristofolin-A (30) were isolated from the flower of *A. kaempferi* [45], sodium aristofolin-A (31), aristofolins B, C, and D (32–34) from the leaves of *A. cucurbitifolia* [46], aristofolin-E (35) from the stem and root of *A. kaempferi* [42], and sodium 7-hydroxy-8-methoxyaristololate (36) from the root and stem of *A. cucurbitifolia* [33], demethylaristofolin E (37) from the stem of *A. manshuriensis* [47], aristolamide (38) from the roots of *A. indica* [48] and aristolamide II (39) from the stem of *A. manshuriensis* [49], sodium 9-hydroxy-10-formyloxy aristolochate I (40) and sodium 7,9-dihydroxy-10-formyloxy aristolochate I (41) from the roots of *A. contorta* [18] (Fig. 2). We have updated the ^1H -NMR spectroscopic data of aristolochic acid (42) and its methyl ester (43), using the experimental data of the corresponding compounds isolated from the Taiwanese butterfly *Pachliopta aristolochiae interpositus*, which exclusively feeds on *A. cucurbitifolia* [50] and the synthetic products [19, 51]. Biogenetically,



- 1** $R_1 = H$ $R_2 = OGlc$ $R_3 = H$ $R_4 = OCH_3$
2 $R_1 = CH_3$ $R_2 = OGlc$ $R_3 = H$ $R_4 = OCH_3$
3 $R_1 = H$ $R_2 = OCH_3$ $R_3 = H$ $R_4 = OCH_3$
4 $R_1 = CH_3$ $R_2 = OCH_3$ $R_3 = H$ $R_4 = H$
5 $R_1 = CH_3$ $R_2 = OCH_3$ $R_3 = H$ $R_4 = OCH_3$
6 $R_1 = CH_3$ $R_2 = H$ $R_3 = H$ $R_4 = OCH_3$
13 $R_1 = H$ $R_2 = OGlc$ $R_3 = H$ $R_4 = H$
14 $R_1 = Na$ $R_2 = H$ $R_3 = H$ $R_4 = OCH_3$
15 $R_1 = Na$ $R_2 = OH$ $R_3 = H$ $R_4 = H$
16 $R_1 = Na$ $R_2 = H$ $R_3 = OH$ $R_4 = OCH_3$
17 $R_1 = Na$ $R_2 = H$ $R_3 = H$ $R_4 = H$
18 $R_1 = Na$ $R_2 = OH$ $R_3 = H$ $R_4 = OCH_3$
19 $R_1 = H$ $R_2 = H$ $R_3 = OH$ $R_4 = OCH_3$
20 $R_1 = CH_3$ $R_2 = H$ $R_3 = OCH_3$ $R_4 = OCH_3$
21 $R_1 = Na$ $R_2 = H$ $R_3 = OCH_3$ $R_4 = OCH_3$
22 $R_1 = CH_3$ $R_2 = H$ $R_3 = H$ $R_4 = OH$
23 $R_1 = H$ $R_2 = OH$ $R_3 = H$ $R_4 = OCH_3$
24 $R_1 = H$ $R_2 = H$ $R_3 = H$ $R_4 = H$
25 $R_1 = H$ $R_2 = OH$ $R_3 = H$ $R_4 = H$
26 $R_1 = H$ $R_2 = H$ $R_3 = OH$ $R_4 = H$
27 $R_1 = H$ $R_2 = OH$ $R_3 = H$ $R_4 = OH$



- 7** $R_1 = CH_3$ $R_2 = H$ $R_3 = H$ $R_4 = H$
8 $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = H$ $R_4 = H$
9 $R_1 = CH_3$ $R_2 = H$ $R_3 = OH$ $R_4 = H$
10 $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = OH$ $R_4 = H$
11 $R_1 = CH_3$ $R_2 = H$ $R_3 = H$ $R_4 = OCH_3$
12 $R_1 = CH_3$ $R_2 = CH_3$ $R_3 = H$ $R_4 = OCH_3$

Fig. 1 Structures of aristolochic acid derivatives

the nitro group of aristolochic acids can be replaced by hydrogen in a reaction called hydrogenolysis. Experimentally, Priestap *et al.* showed evidence for a conversion of aristolochic acid I into aristolic acid on treatment with cysteine or GSH (glutathione) under physiological conditions [19]. Hydrogenolysis may involve the direct transfer of a hydride ion from the thiol group to eliminate the nitro group at C-10 of aristolochic acid I, leading to the denitro derivative.

Not much information is available about the cytotoxicity of derivatives of aristolochic acids. In a few cases when the cytotoxicity of aristolic acid II and aristolic acid is correlated with that of the corresponding AAs, the loss of the nitro group significantly reduces the cytotoxicity of the denitro derivatives of AAs [14].

2.3 Sesqui- and diterpene esters of aristolochic acids

Hybrid structures of sesquiterpene ester of AAs, including aristoloterpenates I, II, III, and IV (44–47) [52] and aristophyllides A, B, C, and D (48–51) [53], were isolated from the root and stem of *A. heterophylla*. *ent*-Kaurane diterpene esters of aristolochic acids, aristolin (52) and aristolins I (16 α -hydroxy-*ent*-17-kauranyl aristolochate I) (53) and II (16 α -hydroxy-*ent*-17-kauranyl aristolochate II) (54), were isolated from the root and stem of *A. elegans* [54] and the tubercula of *A. pubescens* [39], respectively (Fig. 3).

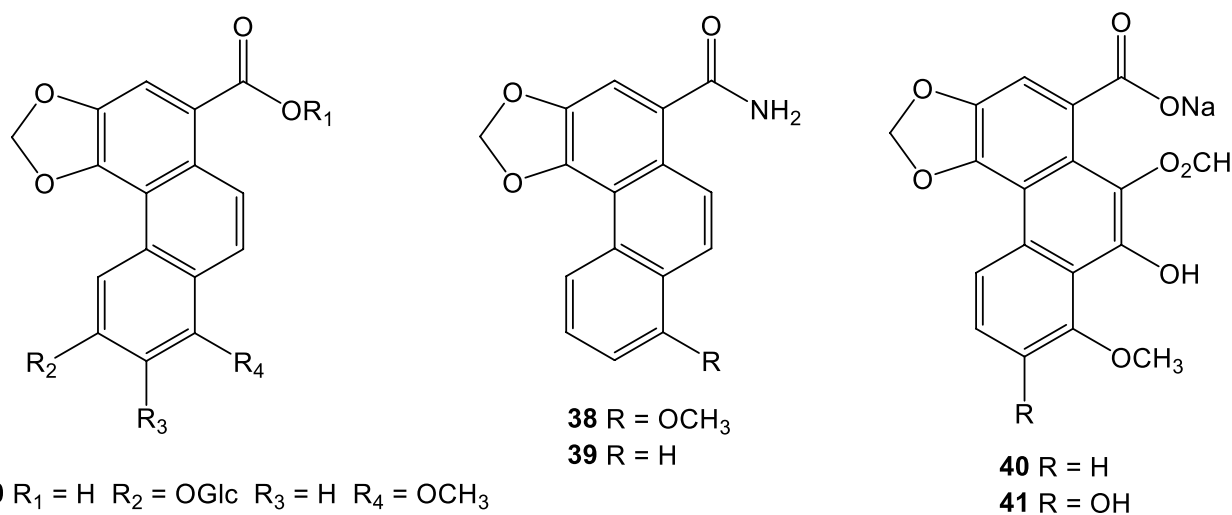


Fig. 2 Structures of denitroaristolochic acid derivatives

3 Analytical and preparative separation of aristolochic acids

High-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector is considered one of the most reliable techniques for the analysis of substances with small quantities in biological samples. As the causative agents of nephropathy, analytical HPLC methods have been developed for the qualitative and quantitative analysis of AAs I and II in *Aristolochia* herbs. The analysis of other AAs and aristolactams based on comparison of HPLC retention time often encounters the shortage problem of reference compounds. PDA detectors are often used with the UV detection wavelength set at 250, 254, or 260 nm. Quantification (w/w) was based on calibration curves, which were constructed by plotting area vs. concentration. In general, simple, rapid, and accurate HPLC methods were developed to enable the simultaneous identification of AAs. Aristolochic acids have been found in almost all the *Aristolochia* samples analyzed in these HPLC studies, and the content of aristolochic acid I is usually higher than that of aristolochic acid II. To our surprise, hardly over twenty *Aristolochia* species have been found to contain AAs, making the

development and validation of the HPLC analytical methods practically crucial [1].

Hashimoto et al. used HPLC with a Waters ODS column and MeOH-1% acetic acid (50:50, v/v) elution to examine AAs I and II in *A. debilis*, *A. fangchi*, and *A. manshuriensis*. The quantitative results showed the levels of AAs I and II in *A. manshuriensis* were higher than the non-toxic effect level established at 0.2 mg/kg [55]. The amounts of AAs I and II in the different parts (root, stem, leaves, fruit) of *A. clematidis* were quantified by Bartha et al. [56]. AAs I and II were found to have the highest contents in the herb's root. Using a non-acidic methanol-water (60:40) solvent system and an ODS Hypersil C18 column, Li et al. observed a reasonable separation of AAs I and II in *A. fangchi* [57]. Many factors, such as collection region, cultivation methods, and parts of the plants, can affect the contents of AAs. Alali et al. used a HPLC LichroCART[®] 125-4 column and a MeOH-1% acetic acid (60:40, v/v) solvent system to identify AAs I and II in root, stem, and leaves of *A. maurorum* [58]. The analytical samples were extracted in these experiments using a mixture of 80% MeOH and 20% formic acid in water. Because the parent substances contained both a carboxyl group and a nitro group, the acidic condition was

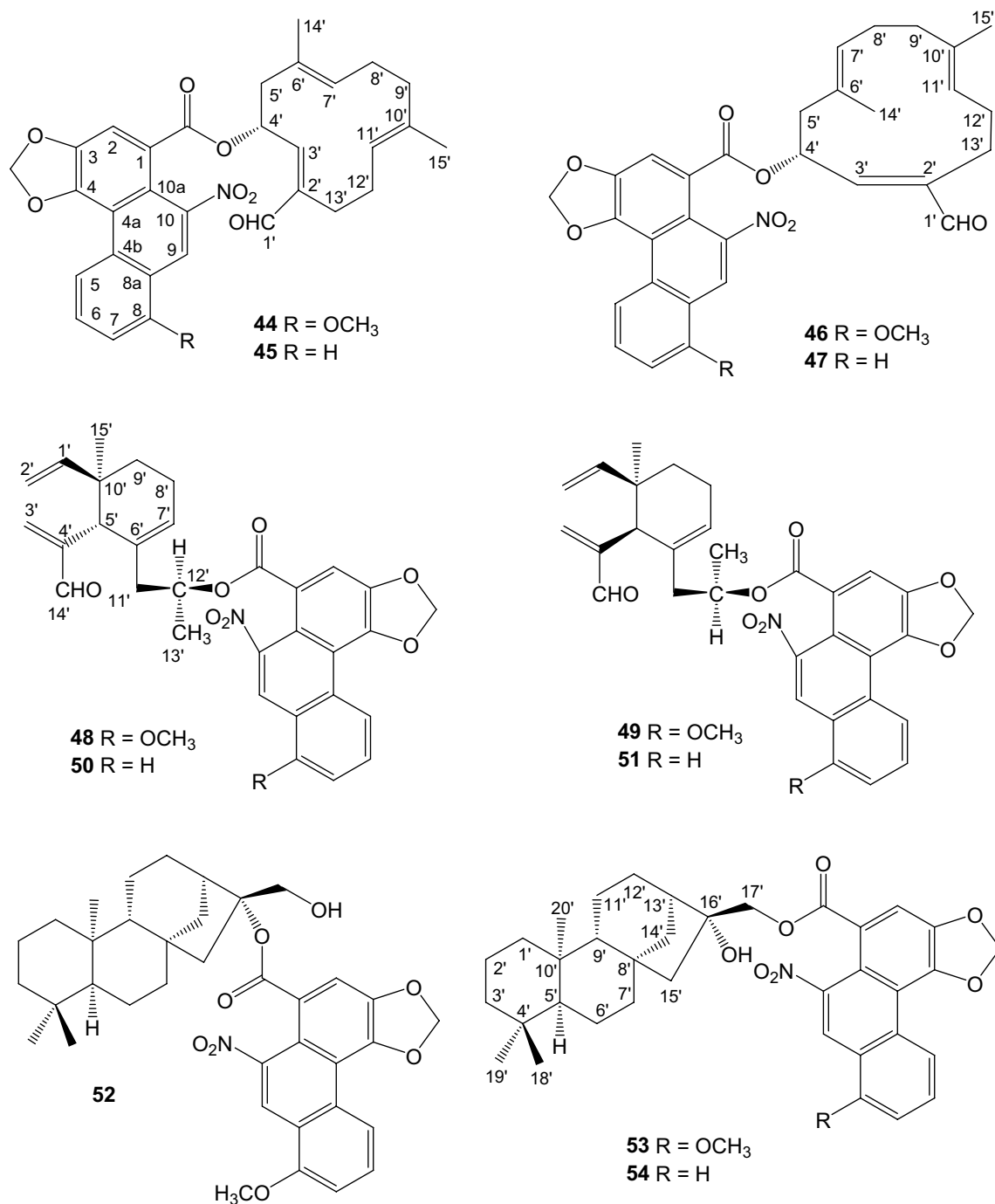


Fig. 3 Structures of sesqui- and diterpene esters of aristolochic acids

considered favorable to extract AAs from plant materials. Root was found to be the main storage of the two AAs during the flowering stage. Using a C₁₈ HPLC method with a Zobrax SB-C₁₈ column, acetonitrile and 3.7 mM phosphoric acid buffer gradient elution, Zhang et al. successfully separated five AAs (aristolochic acid I, II, IVa,

Va, and 9-hydroxyaristolochic acid-1) and two aristolactams (aristolactams I and 2) from the fruit of *A. contorta* (traditional drug name in the *Pharmacopoeia of the People's Republic of China*: Madouling), root of *A. contorta* (Bei-madouling-gen), herbs of *A. contorta* and *A. debilis* (Tianxianteng), root of *A. debilis* (Quingmuxiang),

stem of *A. manshuriensis* (Guanmutong), and root of *A. fangchi* (Guangfangji) [59]. After examining 60 samples, only AAs I and II were detected in the root and herbs of *A. debilis*, while AAs I, II, and IVa were identified in the root of *A. fangchi* and the stem of *A. manshuriensis*. All seven compounds were found in the fruit and herbs of *A. contorta*, with the exception of aristolactam-II, which was absent from the herbs of *A. contorta*. Furthermore, the findings demonstrated that the majority of *Aristolochia* herbs in China and Japan had a significantly higher amount of aristolochic acid I than aristolochic acid II. Using an HPLC method with a Waters C-18 HPLC column, MeOH-1% acetic acid (60:40, v/v), and UV detection at 250 nm, Abdelgadir et al. quantified the amounts of AAs I and II in the whole plant of *A. bracteata* collected in Sudan as 12.98 g/kg and 49.03 g/kg, respectively [60]. This is the first report on the higher amount of aristolochic acid II than that of aristolochic acid I; this could be due to geographic or biological diversity of the *Aristolochia* species studied [60]. Using an Inert-Sustain C18 column and an isocratic solvent mixture of 0.1% acetic acid in the ratio acetonitrile–water (50:50), Araya et al. analyzed AAs I and II in the leaves of *A. chilensis* [61]. To confirm the identification of AAs, each HPLC signal was collected and directly injected into an Orbitrap mass detector; the resulting mass spectra were compared with those of the commercial samples. Rodríguez et al. used ethyl acetate to extract AAs from leaves and stems of *A. sprucei*. They used a Supelco™ LC18 column with a photodiode array detector monitored at 254 nm and a solvent gradient of 10–66% MeOH in water for the first 32 min, followed by 66–10% MeOH for the next 32–35 min, and 100% MeOH for the time left, to analyze the crude extracts [62]. Aristolochic acid I was identified in the stem extract, but not in the leaf extract of *A. sprucei*. The chromatographic condition showed the distinct separation of aristolochic acid I from the other compounds in the extracts.

Metabolomics techniques based on LC–ESI–MS and ^1H -NMR spectroscopy are also used to ascertain the variation of AAs and their derivatives. Michl et al. were able to screen aristolochic acid analogues in 43 medicinally used *Aristolochia* species using this method [30]. Metabolomics data processing involves AMIX software for the ^1H -NMR spectra in the range 0.1–10 ppm and Mzmine 2 for peak extraction, chromatogram deconvolution, and peak alignment in the HR LC–MS data. While most of the compounds were tentatively assigned based on LC–MS accurate mass, retention times, UV maxima, MS/MS fragmentation ions, and ^1H -NMR data set some analogues were identified by comparison with known reference standards. Consequently, AAs I and II were found to be the most prevalent members, and that

carbohydrates and fatty acids are the primary chemical markers that differentiate *Aristolochia* samples. Based on MS data libraries of Traditional Medicine Zhang et al. developed an UP (Ultra-Performance)LC–QTOF–MS/MS method using a Waters Acquity UPLC–BEH C₁₈ column, a gradient elution of 0.1% formic acid aqueous solution–acetonitrile, and ESI–MS detection with a full scale mode ranged m/z 50–1500 [62]. Compounds were identified in three species of *Aristolochia* herbs, including *A. mollissima*, *A. debilis*, and *A. cinnabaria*, by comparing chromatographic and MS characteristics with those of four aristolochic acid standards and the published literature. To explain the qualitative and quantitative variations in the three *Aristolochia* herbs from different origins, the authors employed 19 differential markers of AAs and aristolactams from *A. mollissima*, 16 differential markers from *A. debilis*, and 22 differential markers from *A. cinnabaria*. Compared to the other species of *A. mollissima* and *A. debilis*, *A. cinnabaria* had much higher levels of AAs.

Capillary electrophoresis (CE) offers many advantages, such as high speed, high efficiency, ultra-small sample volume, and low consumption of solvent [63]. Coupling electrochemical detection (ED) with CE can provide high separation efficiency and good sensitivity. Zhou et al. detected AAs I and II in the fruit, stem, and root of *A. debilis* using a 33 μm carbon fiber electrode (CFE) as the working electrode for the [64]. An aqueous buffer with 2.0×10^{-2} mol L⁻¹ phosphate buffer solution (PBS) (pH 10.0) was used to accomplish the separation. In accordance with the results of many previous analytical reports, the contents of AAs in the root are higher than those in the fruit, but abnormally, they were not detected in the stem.

The recent emphasis on qualitative and quantitative investigation of AAs in the original plants or formulations was highlighted by Zhang et al. [65]. The works require high-purity reference compounds. When using preparative liquid chromatography, the required purity of the target compounds is achieved if the distance between zones of compounds under separation increases. Using Waters Auto-purification Factory with an oligo (ethylene glycol) (OEG) separation column to cluster compounds with similar structures and preparative PDA–HPLC with a C-18 separation column to purify the target compounds the authors develop a method for selective purification of AAs from *Aristolochia* plants. A gradient solvent system with 0.2% formic acid water and methanol and 0.2% formic acid water and acetonitrile were used for fraction separation and compound purification, respectively. The detection wavelength was 260 nm. Aristolochic acids from *A. manshuriensis* were successfully separated and purified using this method. Duan et al. used an advanced

counter-current chromatography (CCC) technique, called pH-zone-refining CCC (pH-ZRCCC), in preparative isolation of AAs [66]. A large-scale separation technique for organic acids and bases according to their pH in displacement mode, pH-ZRCCC was introduced in 1991. The method uses a retainer base (or acid) to hold the analytes in the column and a displacer acid (base) to elute the analytes in the decreasing order of pK_a and hydrophobicity. pH-ZRCCC has been shown to be effective in isolating target compounds from the EtOAc extract from the stem of *A. manshuriensis* with high purity in a single run and is not affected by irreversible adsorption. Petroleum ether-EtOAc-MeOH-water (3:7:3:7, v/v/v/v) was the optimum two-phase solvent system. Triethylamine and trifluoroacetic acid were added to the organic phase. The collection was monitored with a UV detector at 254 nm. The elution order of AAs in the chromatogram (aristolochic acid IIIa > aristolochic acid IVa > aristolochic acid II > aristolochic acid I > aristolic acid II > aristolic acid I) was in accordance with the hydrophobic properties of AAs and their acidities.

4 Structure elucidation of aristolochic acids and derivatives

According to the structural features of isolated AAs from the genus *Aristolochia* they have been grouped into the following groups: i) AAs, their sodium salts, and their methyl esters; ii) denitroaristolochic acids (derivatives of aristolic acids), their sodium salts, and their methyl esters; and iii) sesqui- and diterpene esters of AAs. Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 list the ^1H - and ^{13}C -NMR spectroscopic data for AAs, denitroaristolochic acids, and terpene esters of AAs. The nonpolar, aprotic CDCl_3 is used for NMR measurements of less polar methyl esters of AAs, denitroaristolochic acids and terpene esters of AAs, whereas the polar, aprotic $\text{DMSO}-d_6$ is the common NMR solvent for AAs. On a smaller scale, acetone- d_6 is used for methyl esters of AAs and methanol- d_4 is used for polar AA derivatives, such as their glucosides or sodium salts. Due to interactions between NMR solvents and compounds, measured chemical shifts may vary for the same compounds as observed for compound 42 in $\text{DMSO}-d_6$ and acetone- d_6 (Table 8). Since typical structures of AAs are not complex, with most of the signals falling in the downfield region, the resolution of substituted aromatic C-rings from H-5 to H-8 may benefit from high-field NMR techniques. For example, the ^1H -NMR data of compound 42 having an 8-methoxy substituent in $\text{DMSO}-d_6$ are almost coincident when measured at 60 MHz [51] and 400 MHz [19]. Most of the time, 2D NMR techniques such as HSQC (or HMQC), HMBC, and COSY were used to assign the proton and carbon-13 signals.

Aristolochic acids all have structures of 10-nitro-phenanthrenecarboxylic acid. The majority of AAs have a 3,4-methylenedioxy group (phenanthro[3,4-*d*]-1,3-dioxole-10-nitro-1-carboxylic acids). In the IR spectra, the presence of the nitro group is indicated at ν_{max} ca. 1525 and 1346 cm^{-1} , whereas the carbonyl group absorbs in the range 1710–1600 cm^{-1} [38]. At C-6, C-7, or C-8, the C ring of the phenanthrene skeleton is commonly substituted with a hydroxyl or a methoxy group, resulting in monooxygenated, deoxygenated, or tri-oxygenated phenanthrenecarboxylic acids. To confirm the locations of the hydroxyl or methoxy substituents of the C ring, NOESY experiments can be conducted [36]. In some examples, the C-ring is unsubstituted. Taken together, the following characteristics are visible in the ^1H -NMR spectra of most AAs: the singlet signals of aromatic H-2 and H-9 at δ_{H} ca. 7.70–7.80 and 8.20–8.70, respectively, and the proton spin-spin splitting of aromatic H-5 and H-8 in dependency of the aromatic substitution patterns of H-6, H-7, and H-8. C-5 is unsubstituted in all instances, and the proton signal for H-5 is often shifted downfield at δ_{H} ca. 8.20–8.70 because of the shielding effect of the phenanthrene A ring. The methylenedioxy group is indicated by a two-proton singlet signal at δ_{H} ca. 6.40, δ_{C} ca. 102–104. The common derivatives of AAs are their methyl esters [16, 31–33, 35, 40, 42], which may be inferred from an additional methoxy signal at δ_{H} ca. 4.00 in their ^1H -NMR spectra. Comparisons of chemical shifts of the phenanthrene C-ring of 3,4-methylenedioxyaristolochic acids can lead to some remarks about substituent effects. Glucosylation at C-6 of AA I [17] (compound 1, Table 3) causes upfield shifts of H-5 (–0.26 ppm) and H-7 (–0.20 ppm) in $\text{DMSO}-d_6$. The shielding effects observed for 2 are H-5 (–0.16 ppm) and H-7 (–0.34 ppm). Methoxy and hydroxy groups are common substituents of the C-rings of AAs isolated from *Aristolochia* plants. 6-Oxy, 7-oxy, 6,8-dioxy, and 7,8-dioxy are substitution patterns observed in the current review, and the shielding (upfield shift) or deshielding effect (downfield shift) compared with AAII (24) in $\text{DMSO}-d_6$ are summarized in Fig. 4. Occasionally, sodium aristolochates are isolated [38, 41]. Their salt form is indicated by the carboxyl group appears at ν_{max} ca. 1540–1580 cm^{-1} in the IR spectra [38]. In comparison between ^1H - and ^{13}C -NMR spectroscopic data of the free AAs and their sodium salts, the chemical shifts the carboxyl groups are at δ_{C} ca. 168 and C-1 at δ_{C} ca. 121–124, whereas H-9 of the sodium salts shifts upfield to δ_{H} ca. 8.15–8.35 (acids: δ_{H} ca. 8.45–8.60) [28, 29]. Treatment of the salt form with 5% HCl and the solution is purified on a Sephadex LH-20 column eluted successively with H_2O and MeOH affords sodium chloride, which

Table 1 Aristolochic acids and derivatives from *Aristolochia* species

Plant	Plant material	Compound	References
<i>A. manshuriensis</i>	Stems	Aristoloside (1)	[31]
<i>A. longa</i>	Root	Aristolochic acid IV (3)	[32]
<i>A. cucurbitifolia</i>	Root, stems	Aristolochic acid III methyl ester (4)	[33]
<i>A. indica</i>	Root	Methyl aristolochate (6)	[34]
<i>A. liukiuensis</i>	Stems	3-Hydroxy-4-methoxy-10-nitro-	[35]
<i>A. auricularia</i>	Root	phenanthrene-1-carboxylic acid methyl ester (7)	[16]
<i>A. kankauensis</i>	Root, stems	Ariskanins A-E (8–12)	[36]
<i>A. cinnabarina</i>	Root	Aristolochic acid IIIa 6-O- β -D-glucoside (13)	[37]
<i>A. foveolata</i>	Leaves	Sodium aristolochate-I (14)	[38]
		Sodium aristolochate-C (15)	[38]
		Sodium 7-hydroxyaristolochate A (16)	[38]
<i>A. pubescens</i>	Tubercula	Sodium aristolochate I (14)	[39]
		Sodium aristolochate IIIa (15)	[39]
		Sodium aristolochate II (17)	[39]
		Sodium aristolochate IVa (18)	[39]
		Aristolochic acid VIIa (19)	[39]
<i>A. cucurbitifolia</i>	Leaves	Aristolochic acid-VII methyl ester (20)	[40]
<i>A. heterophylla</i>	Root, stems	Sodium aristolochate (14)	[41]
		Sodium aristolochate-VII (21)	[41]
<i>A. kaempferi</i>	Root, stems	Aristolochic acid-Ia methyl ester (22)	[42]
<i>A. bracteolata</i>	Leaves, stems	Aristolochic acid-D (23)	[43]
<i>A. fangchi</i>	Root	Aristolochic acids B, C, F, G (24–27)	[17]
<i>A. championii</i>	Rhizomes	Aristchamic A (28)	[24]
<i>A. contorta</i>	Roots	Aristolochic acid VIIa (19)	[18]
		Aristolochic acid II (24)	[18]
<i>A. maurorum</i>	Whole plant	Aristolochic acid II alanine amide (29)	[44]
<i>A. kaempferi</i>	Flower	Aristofolin-A (30)	[45]
<i>A. cucurbitifolia</i>	Leaves	Sodium aristofolin-A (31)	[46]
		Aristofolins B, C, and D (32–34)	[46]
<i>A. kaempferi</i>	Root, stems	Aristofolin-E (35)	[42]
<i>A. cucurbitifolia</i>	Root, stems	Sodium 7-hydroxy-8-methoxyaristolatate (36)	[33]
<i>A. manshuriensis</i>	Stems	Demethylaristofolin E (37)	[47]
<i>A. indica</i>	Roots	Aristolamide (38)	[48]
<i>A. manshuriensis</i>	Stems	Aristolamide II (39)	[49]
<i>A. contorta</i>	Roots	Sodium 9-hydroxy-10-formyloxy aristolochate I (40)	[18]
		Sodium 7,9-dihydroxy-10-formyloxy aristolochate I (41)	[18]
<i>A. heterophylla</i>	Root, stems	Aristoloterpenates I, II, III, IV (44–47)	[52]
		Aristophyllides A, B, C, D (48–51)	[53]
<i>A. elegans</i>	Root, stems	Aristolin (52)	[54]
<i>A. pubescens</i>	Tubercula	Aristoloin I (53)	[39]
		Aristoloin II (54)	

is determined by atomic absorption spectrometry [33, 38, 41]. The MeOH solution affords free acids, whose carbonyl group appears at $\nu_{\max}$ ca. 1660–1710 cm^{-1} in the IR spectra [38]. Compared to those of the free acids H-9 of the sodium salts of AAs appeared slightly upfield at δ_{H} ca 8.15–8.35 [38].

Aristolochic acids can be converted into denitro compounds by reduction, both chemically [20, 50] and biologically [19], e.g., aristolochic acid I is converted to aristolic acid. Similar to the nitro compounds, the

occurrence of denitroaristolochic acids as their methyl esters [34] or sodium salts [45] is frequently found in *Aristolochia* herbs. In one publication [47], primary amide derivatives of aristolic acids were isolated; the amide group was identified by an IR absorption band for a NH_2 group at $\nu_{\max}$ 3182 cm^{-1} and a carbon-13 signal at δ_{C} 170.1. Free carboxylic acids are identified by an IR absorption band for the carboxyl group at $\nu_{\max}$ 3000 and 1670 cm^{-1} and a carbon-13 signal at δ_{C} ca. 168.0 [46]. The presence of the carboxyl group can be detected by

Table 2 ¹H- and ¹³C-NMR characteristic signals of aristolochic acid derivatives (δ in ppm, J in Hz, DMSO-*d*₆)

H/C	3,4-Methylenedioxyaristolochic acids		3,4-Methylenedioxyaristolochic acid	
	¹ H-NMR	¹³ C-NMR	¹ H-NMR	¹³ C-NMR
2	ca. 7.70–7.80 s	ca. 111–114	ca. 7.70–7.80 s	ca. 111–114
5	ca. 8.20–8.70 s	ca. 111–130	ca. 8.20–8.90 s	ca. 111–130
9	ca. 8.20–8.70 s	ca. 120–130	ca. 8.80 d (9.0–10.0)	ca. 124–127
10		ca. 143–146	ca. 7.70 d (9.0–10.0)	ca. 124–127
O-CH ₂ -O	ca. 6.40 s	ca. 102–104	ca. 6.40 s	ca. 102–104
OCH ₃	ca. 4.0 s	ca. 56–61	ca. 4.0 s	ca. 56–57

D₂O exchange. Al-Barham et al. reported the isolation of a secondary amide of aristolochic acid (**29**) [44], which has an IR absorption band for the NH group at $\nu_{\max}$ 3466 cm⁻¹ and an amide carbon-13 signal at δ_C 174.5. In addition, the NH signal in the ¹H-NMR spectrum appears at δ_C 8.97 (*br s*). In accordance with the structural change to phenanthrene-1-carboxylic acids, the NMR spectra of the denitro derivatives show signals of a *cis*-configured C-9/C-10 double bond at δ_H ca. 8.80 and 7.70 (*J* ~ 9–10 Hz) and δ_C ca. 124–127. Glucosylation at C-6 of aristolochic acid (**42**) (compound **31**, Table 6) causes upfield shifts of

H-5 (-0.35 ppm) and H-7 (-0.28 ppm) in DMSO-*d*₆. The shielding or deshielding effects of 8-oxy, 7,8-dioxy, and 6,8-dioxy substituents in DMSO-*d*₆ compared with aristolochic acid (**42**) are summarized in Fig. 5. Two first formyloxy derivatives of aristolochic acids are reported in [18] with C-9 and C-10 substituted with a hydroxyl group and a formyloxy group, respectively. Consequently, the olefinic carbon/proton signals are not observed, instead, the formyloxy group resonances at δ_H ca. 8.20 and at δ_C ca. 162. Since the compounds occurred as their sodium salts, the cation portion was confirmed by ICP-MS.

Species of *Aristolochia* have been reported to contain elemene, caryophyllene, and humulene [12]. Mandolin R, mandolin L, mandolin M, and arystophyllene [12], which were previously isolated from the root and stem of *A. heterophylla*, may be related precursors for the sesquiterpene alcohol moieties of aristolochic acid esters from *Aristolochia* species, however, more research is needed to identify the direct precursor compounds. *ent*-Kaurane diterpenoids are widely isolated from *Aristolochia* species, including *A. elegans* [12]. Epoxide ring-opening of the precursor 16,17-epoxy-*ent*-kaurane or by an esterification of AAs and *ent*-kaurane-16,17-diol may produce aristolins (**52**), aristolins I (**53**) and II (**54**). These terpeno-aristolochic acid hybrid compounds are identified by ¹³C-NMR, which revealed 14 carbons for the core phenanthrene moiety, a carboxyl group, and 15 or 20

Table 3 ¹H-NMR spectroscopic data of compounds **1–7** (δ in ppm, J in Hz)

H	1 ^{a&} [31] 90 MHz	2 ^b [31] 200 MHz	3 ^a [32] 60 MHz	4 ^{c%} [33] 200 MHz	5 ^c [32] 200 MHz	6 ^a [34] 60 MHz	7 ^a [16] 250 MHz	7 ^d [35] 270 MHz
2	7.78 s	7.73 s	7.70 s	7.76 s	7.73 s	7.80 s	7.80 s	7.83 s
5	8.35 d (2.0)	8.45 dd (2.1, 0.6)	7.84 d (2.0)	8.60 d (2.4)	8.13 d (2.0)	8.66 d (8.1)	9.60 d (8.6)	9.60 <i>br d</i> (8.7)
6						7.68 t (7.7)	7.80 t (8.1)	7.82 <i>m</i>
7	7.13 d (2.0)	6.99 d (2.1)	6.78 d (2.0)	7.34 dd (8.8, 2.4)	6.70 d (2.0)	7.28 d (8.1)	7.91 t (8.1)	7.73 <i>m</i>
8				7.90 d (8.8)			8.26 d (8.1)	8.15 dd (6.2, 1.3)
9	8.50 s	8.73 d (0.6)	8.32 s	8.31 s	8.74 s	8.36 s	8.59 s	8.41 s
O-CH ₂ -O	6.49 s 6.44 s	6.40 d (1.2) 6.37 d (1.2)	6.39 s	6.39 s	6.34 s	6.43 s		
OCH ₃	4.07 s	4.06 s	3.97 s 3.88 s	4.02 s	3.98 s 4.01 s	4.05 s	3.93 s	3.93 s*
CO ₂ Me		3.86 s		3.87 s	3.86 s	3.81 s	3.73 s	3.83 s*

^a DMSO-*d*₆

^b CDCl₃-CD₃OD

^c CDCl₃

^d (CD₃)₂CO

^e CD₃OD

* interchangeable

& Glc: 5.12 d (7.0), 3.2–4.2 *m*

% Glc: 5.15 d (7.3), 3.98 dd (12.0, 2.0), 3.81 dd (12.0, 5.0), 3.45–3.70 *m*

% OH: 9.47 s

Table 4 ¹H-NMR spectroscopic data of compounds **8–16** (δ in ppm, *J* in Hz)

H	8 ^c [36] unknown	9 ^{d&} [36] unknown	10 ^{d\$} [36] unknown	11 ^{c%} [36] unknown	12 ^c [36] unknown	13 ^{e^} [37] unknown	14 ^a [38] unknown	15 ^a [38] unknown	16 ^{a*} [38] unknown
2	7.89 <i>s</i>	7.76 <i>s</i>	7.89 <i>s</i>	7.89 <i>s</i>	7.88 <i>s</i>	7.68 <i>s</i>	7.62 <i>s</i>	7.69 <i>s</i>	7.70 <i>s</i>
5	9.66 <i>dd</i> (8.0, 1.8)	9.13 <i>d</i> (2.3)	9.21 <i>d</i> (2.3)	8.99 <i>d</i> (8.8)	9.21 <i>d</i> (8.0)	8.88 <i>d</i> (2.5)	8.63 <i>d</i> (8.2)	8.43 <i>d</i> (2.2)	8.65 <i>d</i> (9.0)
6	7.81 <i>td</i> (8.0, 1.6)			7.74 <i>t</i> (8.8)	7.72 <i>d</i> (8.0)		7.74 <i>t</i> (8.2)		7.48 <i>d</i> (9.0)
7	7.71 <i>td</i> (8.0, 1.8)	7.34 <i>dd</i> (8.5, 2.3)	7.34 <i>dd</i> (8.7, 2.3)	7.13 <i>d</i> (8.8)	7.11 <i>d</i> (8.0)	7.48 <i>dd</i> (8.9, 2.5)	7.27 <i>d</i> (8.2)	7.21 <i>dd</i> (8.4, 2.2)	
8	7.99 <i>dd</i> (8.0, 1.6)	8.08 <i>d</i> (8.5)	8.07 <i>d</i> (8.7)			7.99 <i>d</i> (8.9)		7.98 <i>d</i> (8.4)	
9	8.34 <i>s</i>	8.38 <i>s</i>	8.40 <i>s</i>	8.87 <i>s</i>	8.89 <i>s</i>	8.23 <i>s</i>	8.30 <i>s</i>	8.18 <i>s</i>	8.26 <i>s</i>
O-CH ₂ -O						6.34 <i>s</i> , 6.37 <i>s</i>	6.35 <i>s</i>	6.36 <i>s</i>	6.37 <i>s</i>
OCH ₃	3.97 <i>s</i> 4.10 <i>s</i>	3.93 <i>s</i>	3.99 <i>s</i> 4.10 <i>s</i>	3.89 <i>s</i> 4.08 <i>s</i>	3.94 <i>s</i> 4.05 <i>s</i> 4.10 <i>s</i>		4.03 <i>s</i>		3.95 <i>s</i>
CO ₂ Me	3.88 <i>s</i>	3.76 <i>s</i>	3.77 <i>s</i>	3.85 <i>s</i>	3.88 <i>s</i>				

^a DMSO-*d*₆

^c CDCl₃

^d (CD₃)₂CO

^e CD₃OD

[&] OH: 9.29 *s*, 9.52 *s*

^{\$} OH: 9.47 *s*

[%] OH: 6.42 *s*

[^] Glc: 5.14 *d* (6.9), 3.95 *dd* (11.9, 2.0), 3.80 *dd* (11.9, 5.4), 3.58 *m*, 3.50 *d* (11.9)

^{*} OH: 10.50 *brs*

Table 5 ¹H-NMR spectroscopic data of compounds **19–28** (δ in ppm, *J* in Hz)

H	19 ^{a^} [18] 500 MHz	20 ^c [40] 200 MHz	21 ^a [41] 200 MHz	22 ^c [42] 200 MHz	23 ^a [43] 400 MHz	24 ^a [17] 500 MHz	25 ^{a&} [17] 500 MHz	26 ^{a#} [17] 500 MHz	27 ^{a\$} [17] 500 MHz	28 ^c [24] 500 MHz
2	7.70 <i>s</i>	7.70 <i>s</i>	7.71 <i>s</i>	7.73 <i>s</i>	7.69 <i>s</i>	7.75 <i>s</i>	7.73 <i>s</i>	7.72 <i>s</i>	7.76 <i>s</i>	7.49 <i>s</i>
5	8.70 <i>d</i> (9.05)	8.85 <i>d</i> (9.6)	8.80 <i>d</i> (9.5)	8.70 <i>d</i> (8.6)	8.06 <i>d</i> (2.4)	8.52 <i>m</i>	8.42 <i>s</i>	8.69 <i>d</i> (8.2)	8.13 <i>d</i> (2.0)	7.38 <i>d</i> (2.3)
6	7.50 <i>d</i> (9.05)	7.46 <i>d</i> (9.6)	7.74 <i>d</i> (9.5)	7.71 <i>t</i> (8.6)		7.70 <i>m</i>		7.31 <i>dd</i> (8.2, 2.2)		
7				7.10 <i>d</i> (8.6)	6.84 <i>d</i> (2.4)	7.74 <i>m</i>	7.23 <i>d</i> (8.0)		6.72 <i>d</i> (2.0)	6.47 <i>d</i> (2.3)
8						7.90 <i>m</i>	8.02 <i>d</i> (8.0)	7.42 <i>d</i> (2.2)		
9	8.40 <i>s</i>	8.67 <i>s</i>	8.41 <i>s</i>	8.82 <i>s</i>	8.36 <i>s</i>	8.39 <i>s</i>	8.42 <i>s</i>	8.21 <i>s</i>	8.70 <i>s</i>	2.81 <i>dd</i> (17.6, 5.4) 4.23 <i>dd</i> (17.6, 2.4)
10	6.45 <i>s</i>									6.67 <i>d</i> (2.4, 5.4)
O-CH ₂ -O	7.70 <i>s</i>	6.37 <i>s</i>	6.46 <i>s</i>	6.38 <i>s</i>	6.40 <i>s</i>	6.41 <i>s</i>	6.345 <i>s</i>	6.43 <i>s</i>	6.50 <i>s</i>	6.13 <i>d</i> (1.4) 6.28 <i>d</i> (1.4)
OCH ₃	3.98 <i>s</i>	4.04 <i>s</i> 4.06 <i>s</i>	3.98 <i>s</i> 4.00 <i>s</i>		3.98 <i>s</i>					
CO ₂ Me		3.87 <i>s</i>		4.06 <i>s</i>						
CO ₂ H						10.96 <i>s</i>	10.88 <i>s</i>	10.90 <i>s</i>	10.82 <i>s</i>	

^a DMSO-*d*₆

^c CDCl₃

[^] OH: 10.44 *s*

[&] OH: 11.36 *s*

[#] OH: 11.80 *s*

^{\$} OH: 11.36 *s*, 11.50 *s*

carbons for the sesqui- or diterpene moiety, respectively. Compounds **52–54** have been included as *Aristolochia* diterpenes in the review [13]. The HMBC

correlations between the α-proton of the terpene alcohol moiety and the carbonyl group of AAs simplify the identification of the ester linkage. The ester itself

Table 6 ^1H -NMR spectroscopic data of compounds **29–36** (δ in ppm, J in Hz)

H	29 ^{a#} [44] 500 MHz	30 ^{e§} [45] 400 MHz	31 ^{a%} [46] 200 MHz	32 ^{d&} [46] 200 MHz	33 ^c [46] 200 MHz	34 ^e [46] 200 MHz	35 ^c [42] 200 MHz	36 ^e [33] 400 MHz
2	7.64 <i>s</i>	7.47 <i>s</i>	7.51 <i>s</i>	7.89 <i>s</i>	7.83 <i>s</i>	7.78 <i>s</i>	7.90 <i>s</i>	7.31 <i>s</i>
5	9.11 <i>d</i> (8.4)	8.29 <i>d</i> (1.4)	8.30 <i>d</i> (1.4)	8.78 <i>d</i> (9.3)	8.84 <i>d</i> (9.2)	8.16 <i>d</i> (2.4)	9.11 <i>m</i>	8.64 <i>d</i> (9.1)
6	7.90 <i>t</i> (7.8)			7.31 <i>d</i> (9.3)	7.32 <i>d</i> (9.2)		7.63 <i>m</i>	7.12 <i>d</i> (9.1)
7	7.80 <i>t</i> (7.6)	6.93 <i>d</i> (1.4)	6.92 <i>d</i> (1.4)			6.70 <i>d</i> (2.4)	7.63 <i>m</i>	
8	8.24 <i>d</i> (7.8)						7.87 <i>m</i>	
9	8.52 <i>s</i>	7.69 <i>d</i> (9.4)	7.77 <i>d</i> (9.4)	7.96 <i>d</i> (9.7)	8.05 <i>d</i> (9.8)	7.96 <i>d</i> (9.4)	7.71 <i>d</i> (9.6)	7.78 <i>d</i> (9.5)
10		8.77 <i>d</i> (9.4)	8.80 <i>d</i> (9.4)	8.96 <i>d</i> (9.7)	8.82 <i>d</i> (9.8)	8.50 <i>d</i> (9.4)	8.85 <i>d</i> (9.6)	8.27 <i>d</i> (9.5)
O-CH ₂ -O	6.52 <i>t</i> (4.2)	6.25 <i>s</i> 6.19 <i>s</i>	6.30 <i>s</i> 6.24 <i>s</i>	6.41 <i>s</i>	6.32 <i>s</i>	6.31 <i>s</i>	6.35 <i>s</i>	6.20 <i>s</i>
OCH ₃		3.96 <i>s</i>	3.97 <i>s</i>	3.95 <i>s</i>	3.99 <i>s</i> , 4.01 <i>s</i> , 4.03 <i>s</i>	4.00 <i>s</i>		3.92 <i>s</i>
CO ₂ Me							4.00 <i>s</i>	
CONH	8.97 <i>br s</i>							

^a DMSO-*d*₆^c CDCl₃^d (CD₃)₂CO^e CD₃OD[^] OH: 10.44 *s*[#] Ala: 4.36 *s*, 1.42 *d* (7.3)[§] OH: 5.38 *br s*, 4.99–5.19 *m*, 4.64 *m*, Glc: 5.11 *d* (6.0), 3.22–3.73 *m*[%] OH: 5.39 *br s*, 4.90–5.20 *m*, 4.64 *t* (5.0), Glc: 5.08 *d* (5.6), 3.22–3.76 *m*[&] OH: 8.61 *br s*

exhibits an IR absorption band at ν_{max} 1710 cm^{-1} . However, when the ester group is located at tertiary C-16 of the *ent*-kaurane-16 β ,17-diol [12], the upfield shifts of kaurane C-13 and C-17 (δ_{C} *ca.* 43.4 and 63.5 in CDCl₃, respectively) are indicative for the ester location. Kaurane C-16 is anticipated to undergo a downfield shift, e.g., the carbon-13 chemical shift was at δ_{C} 98.1 for *ent*-kaurane C-16 of **52** [39]. Compounds **44–47** possess several chiral centers of the *ent*-elemene moiety. Their absolute stereostructures were determined by using the CD (Circular Dichroism) exciton chirality method [52]. The experimental positive and negative Cotton effects were compared with the chiral exciton couplings between the cyclohexene double bond and the unsaturated aldehyde chromophore (at *ca.* 220 nm) and between the ring double bond and the 3,4-methylenedioxybenzoate chromophore (at *ca.* 260 nm).

The ^{13}C -NMR of compounds **45** and **47** could not be achieved due to small amounts of the isolated samples [52].

5 Conclusion

More aristolochic acid derivatives have been isolated and reported since the early assessment of a few reviews on NMR spectroscopic data of common *Aristolochia* phenanthrene derivatives [27–29]. This review provides an update list of NMR spectroscopic data of AAs isolated from *Aristolochia* herbs. The NMR characteristics of three classes of derivatives, AAs, denitroaristolochic acids, and esters of AAs, are also been briefly discussed. By comparing NMR data, the data would facilitate the identification of the bioactive and toxic AAs in *Aristolochia* herbs. In addition, the latest development in

Table 7 ¹³C-NMR spectroscopic data of compounds **13**, **15**–**19**, **23**–**29** (δ in ppm)

C	13 [#] [37]	15 ^a [38]	16 ^a [38]	17 ^a [39]	18 ^a [39]	19 ^a [39]	23 ^b [43]	24 ^a [17]	25 ^a [17]	26 ^b [17]	27 ^a [17]	28 ^c [24]	29 ^d [44]
	Unknown	126 MHz	Unknown	126 MHz	126 MHz	126 MHz	100 MHz	125 MHz	125 MHz	125 MHz	125 MHz	125 MHz	125 MHz
1	123.5	– ^f	124.1	– ^f	– ^f	124.2	118.0	124.6	124.3	123.6	124.3	124.6	128.1
2	112.1	111.6	110.8	114.2	111.5	111.0	112.6	113.8	112.6	112.1	113.9	109.9	111.8
3	145.1	145.3	145.8	145.6	145.3	145.9 [*]	145.7	147.3	146.3	146.3	146.4	148.1	146.9
4	146.7	– ^f	146.3	142.5	143.9	147.0 [*]	145.7	146.8	146.8	147.5	146.8	149.1	146.1
4a	116.3	– ^f	117.8	– ^f	116.4	117.9	117.0	117.3	117.6	118.3	117.4	119.4	117.8
4b	131.0	131.0	132.7	128.9	131.7	131.7	132.1	130.2	133.2	136.9	135.0	130.5	129.3
5	111.4	111.1	121.9	126.4	103.6	121.6 [*]	104.1	127.8	112.0	129.8	115.6	104.1	127.0
6	157.9	159.2	122.8	129.3	160.3	122.9 [*]	158.4	126.1	160.7	110.3	150.8	159.8	130.7
7	95.3	118.2	148.4	127.8	99.2	149.0	100.0	128.0	119.6	159.4	106.9	98.9	129.0
8	135.4	131.7	148.9	129.8	157.6	149.0	161.5	129.8	131.8	116.4	152.4	157.6	130.7
8a	122.5	122.6	122.8	129.0	112.2	122.0	112.3	135.5	122.4	126.3	120.3	111.9	129.3
9	130.3	– ^f	121.5	122.5	117.4	119.2	112.6	121.6	127.2	124.8	121.8	26.5	125.8
10	142.6	– ^f	142.9	148.6	145.1	142.9	145.7	146.0	144.3	145.6	146.3	78.8	145.4
10a	117.6	– ^f	115.5	116.8	– ^f	115.6	117.0	118.3	118.3	118.4	118.6	126.2	116.5
C=O	169.9	– ^f	– ^f	– ^f	– ^f	168.2	168.9	169.6	168.8	168.9	169.8	166.6	167.4
O-CH ₂ -O	101.7	101.9	102.6	101.9	101.7	102.7	102.7	103.1	103.7	103.2	103.8	102.4	103.4
OCH ₃			60.9		56.0	61.0	56.6						

^a DMSO-*d*₆

^c CDCl₃

^f data not shown [24]

[#] Glc: 100.6, 73.0, 77.3, 69.5, 76.6, 60.5

[§] Ala: 174.5, 48.4, 17.7

^{*} interchangeable

Table 8 ¹H- and ¹³C-NMR spectroscopic data of compounds **37**, **39–43** (δ in ppm, J in Hz)

H/C	37 ^a [47] 400 MHz	37 ^a [47] 100 MHz	39 ^a [49] 600 MHz	39 ^a [49] 150 MHz	40 ^a [18] 500 MHz	40 ^a [18] 125 MHz	41 ^a [18] 500 MHz	41 ^a [18] 125 MHz	42 ^a [51] 60 MHz	42 ^d [50] 200 MHz	42 ^a [19] 100 MHz	43 ^c [51] 60 MHz
1		122.3			129.5	128.0		128.8			119.5	
2	7.88 s	112.1	7.78 s		108.9	108.5	7.41 s	108.3		7.98 s	111.7	7.74 s
3		144.6			143.9	143.9		146.1			144.3	
4		146.7			144.2	146.1		146.7			145.6	
4a		115.8			115.6	117.1		115.5			115.5	
4b		128.1			126.6	128.0		128.8			128.4	
5	9.05 m	126.8	9.01 m		126.7	119.6	7.16 d (8.8)	116.1	8.65 d (8.0)	8.73 d (8.6)	118.7	8.70 d (8.2)
6	7.68 m	127.0	7.66 m		127.3	128.4	7.36 d (8.75)	111.9	7.62 t (7.2)	7.61 dd (8.6, 8.2)	127.1	7.58 t (8.1)
7	7.68 m	127.7	7.66 m		127.3	109.0	7.44 d (6.8)	147.0	7.20 d (7.8)	7.21 d (8.2)	107.3	7.01 d (7.5)
8	7.97 m	128.1	7.96 m		127.9	153.9		149.9			154.7	
8a		131.4			131.5	115.3		119.8			121.8	
9	7.79 d (9.6)	127.0	7.70 d (9.0)		125.5	161.5		161.9	8.06 d (9.9)	8.16 d (9.8)	119.1	8.20 d (9.8)
10	8.80 d (9.6)	124.1	8.17 d (9.0)		124.3	145.8		144.7	8.81 d (9.8)	8.97 d (9.8)	123.9	8.80 d (9.7)
10a		127.7			125.2	118.6		118.6			128.0	
C=O		168.4			170.1	171.6		172.0			168.4	
O-CH ₂ -O	6.44 s	102.5	6.39 s		101.9	102.1	6.27 s	101.6	6.42 s	6.44 s	102.5	6.28 s
OCH ₃												
CO ₂ Me						56.3	4.09 s	57.1	4.01 s	4.05 s	55.7	4.01 s
OCHO												3.97 s

^a DMSO-*d*₆

^c CDCl₃

^d (CD₃)₂CO

^e OH: 13.13 br s

Table 9 ¹H- and ¹³C-NMR spectroscopic data of compounds **44–49** (δ in ppm, J in Hz, CDCl₃)

H/C	44 [52] 400 MHz	44 [52] 100 MHz	45 [52] 400 MHz	46 [52] 400 MHz	46 [52] 100 MHz	47 [52] 400 MHz	48 [53] 400 MHz	48 [53] 100 MHz	49 [53] 400 MHz	49 [53] 100 MHz
1		123.2			123.3			124.1		124.0
2	7.87 s	112.7	7.73 s	7.70 s	112.8	7.70 s	7.72 s	112.7	7.76 s	112.8
3		145.8			145.9			145.9		145.9
4		146.6			146.6			146.3		146.4
4a		118.3			118.4			118.4		118.4
4b		130.8			130.8			130.7		130.8
5	8.58 d (8.8)	118.9	9.13 d (8.4)	8.68 dd (8.5, 3.6)	119.1	9.13 d (8.4)	8.64 d (8.0)	119.1	8.68 d (8.2)	119.1
6	7.64 t (8.8)	130.9	7.79 t (8.4)	7.71 t (8.5)	131.0	7.81 td (8.4, 2.4)	7.68 t (8.0)	130.7	7.70 t (8.2)	130.8
7	7.04 d (8.8)	107.8	7.70 t (8.4)	7.10 dd (8.5, 3.6)	107.9	7.71 td (8.4, 2.4)	7.07 d (8.0)	107.8	7.10 d (8.2)	107.8
8		156.7	8.00 d (8.4)		156.9	7.99 dd (8.4, 2.4)		156.8		156.8
8a		119.9			120.1			120.1		120.2
9	8.79 s	121.2	8.35 s	8.84 s	121.3	8.34 s	8.79 s	120.8	8.81 s	120.9
10		145.5			145.6			145.7		145.9
10a		118.1			118.2			118.3		118.4
O-CH ₂ -O	6.34 s	102.4	6.40 s	6.36 s	102.4	6.40 s	6.35 d (1.2) 6.36 d (1.2)	102.3	6.36 d (1.5) 6.37 d (1.5)	102.3
CO ₂ H		166.0			165.8			166.3		166.2
OCH ₃	4.03 s	55.9		4.06 s	55.9		4.05 s	55.9	4.05 s	55.9
1'	10.25 s	191.2	10.25 s	9.51 s	195.9	9.52 s	5.81 dd (17.6, 10.8)	145.5	5.86 dd (17.5, 10.8)	145.6
2'		141.7			145.8		4.79 d (10.8) 4.98 d (17.6)	111.9	5.00 d (17.5) 4.94 d (10.8)	111.9
3'	6.08 d (11.2)	144.4	6.08 d (10.8)	6.36 d (10.6)	148.9	6.36 d (9.6)	6.19 s, 6.24 s	137.4	6.23 s	137.3
4'	6.37 dd (11.2, 4.6)	67.6	6.37 m	5.70 td (10.6, 3.6)	71.6	5.72 td (9.6, 3.4)		150.6		150.5
5'	2.72 br d (12.2) 2.39 br d (12.2)	44.9	2.73 br d (11.4) 2.40 br d (11.4)	2.80 dd (10.6, 3.6) 2.43 t (10.6)	43.6	2.81 dd (12.8, 3.4) 2.43 dd (12.8, 9.6)	3.42 s	43.5	3.43 s	43.4
6'		128.5			129.1			133.4		133.6
7'	5.04 br d (11.2)	130.6	5.05 br d (11.2)	5.10 t (7.0)	129.5	5.11 t (7.6)	5.77 m	126.0	5.71 m	125.7
8'	2.30 br t (11.2) 1.98 br t (11.2)	25.0	2.32 br t (11.2) 2.00 br t (11.2)	2.14 t (7.0)	24.9	2.15 t (7.6)	2.11 br s	22.8	2.09 m	22.9
9'	2.02 m, 2.20 m	39.6	2.03 m, 2.18 m	2.05 m	38.4	2.06 m	1.36 m	27.9	1.42 m	28.0
10'		134.9			133.6			38.3		38.3
11'	4.81 br d (8.4)	125.2	4.82 br d (8.4)	4.85 t (7.8)	125.5	4.85 t (7.6)	2.33 dd (14.4, 8.0) 1.95 br d (14.4)	42.3	2.30 dd (14.0, 5.3) 2.09 m	42.5
12'	2.07 m, 2.42 m	25.9	2.06 m, 2.42 m	2.21 m, 2.33 m	25.1	2.22 m, 2.32 m	5.17 m	71.1	5.09 q (6.5)	71.7
13'	2.84 dt (13.0, 4.0) 1.80 td (13.0, 4.0)	31.7	2.85 br d (12.4) 1.80 br t (12.4)	2.63 dd (9.8, 5.1) 2.40 t (9.8)	25.6	2.64 dd (9.7, 4.9) 2.40 t (9.7)	1.38 d (6.0)	20.1	1.34 d (6.5)	19.7
14'	1.61 s	15.9	1.61 s	1.68 s	18.6	1.69 s	9.63 s	194.2	9.66 s	194.2

Table 9 (continued)

H/C	44 [52] 400 MHz	44 [52] 100 MHz	45 [52] 400 MHz	46 [52] 400 MHz	46 [52] 100 MHz	47 [52] 400 MHz	48 [53] 400 MHz	48 [53] 100 MHz	49 [53] 400 MHz	49 [53] 100 MHz
15'	1.34 s	14.9	1.34 s	1.40 s	15.4	1.41 s	0.71 s	25.9	0.75 s	25.4

^f data not observed [39]

Table 10 ^1H - and ^{13}C -NMR spectroscopic data of compounds **50–54** (δ in ppm, J in Hz, CDCl_3)

H	50 [53] 400 MHz	51 [53] 400 MHz	52 [#] [54] 400 MHz	52 [54] 100 MHz	53 [39] 500 MHz	53 [39] 126 MHz	54 [39] 500 MHz	54 [39] 126 MHz
1				125.0		– ^f		–
2	7.73 <i>s</i>	7.77 <i>s</i>	7.59 <i>s</i>	112.2	7.75 <i>s</i>	112.7	7.76 <i>s</i>	112.8
3				146.3		143.1		– ^f
4				146.8		147.5		146.8
4a				118.4		– ^f		– ^f
4b				130.8		131.0		– ^f
5	9.12 <i>d</i> (8.0)	9.13 <i>d</i> (8.3)	8.61 <i>d</i> (8.0)	119.1	8.72 <i>d</i> (7.5)	119.2	9.17 <i>d</i> (8.0)	127.4
6	7.79 <i>t</i> (8.0)	7.79 <i>t</i> (8.3)	7.64 <i>t</i> (8.0)	130.9	7.74 <i>dd</i> (7.5, 8.0)	131.0	7.83 <i>t</i> (8.0)	130.5
7	7.70 <i>t</i> (8.0)	7.73 <i>t</i> (8.3)	7.04 <i>d</i> (8.0)	107.9	7.13 <i>d</i> (8.0)	108.0	7.74 <i>t</i> (8.0)	– ^f
8	7.97 <i>d</i> (8.0)	7.98 <i>d</i> (8.3)		156.9		156.9	8.01 <i>d</i> (7.5)	130.2
8a				120.1		120.2		128.5
9	8.31 <i>s</i>	8.33 <i>s</i>	8.73 <i>s</i>	120.9	8.86 <i>s</i>	121.2	8.36 <i>s</i>	126.5
10				145.8		145.9		– ^f
10a				118.2		– ^f		118.3
O-CH ₂ -O	6.38 <i>s</i> , 6.40 <i>s</i>	6.39 <i>d</i> (1.4), 6.40 <i>d</i> (1.4)	6.30 <i>s</i>	102.4	6.39 <i>s</i>	102.4	6.42 <i>s</i>	103.0
CO ₂ H				167.3		167.2		167.5
OCH ₃			3.98 <i>s</i>	55.9	4.08 <i>s</i>	56.2		
1'	5.80 <i>dd</i> (17.6, 11.2)	5.85 <i>dd</i> (17.6, 10.8)		42.0	0.76 <i>dt</i> (13.0, 3.0) 1.80 <i>brd</i> (13.0)	40.3	0.76 <i>m</i> 1.80 <i>m</i>	40.4
2'	4.99 <i>d</i> (17.6) 4.79 <i>d</i> (11.2)	5.00 <i>dd</i> (17.6, 0.8) 4.91 <i>dd</i> (10.8, 0.8)		18.3	1.42 <i>m</i>	18.6	1.40 <i>m</i>	– ^f
3'	6.25 <i>s</i> , 6.20 <i>s</i>	6.24 <i>s</i>		42.0	1.15 <i>dt</i> (13.5, 4.0), 1.64 <i>m</i>	41.9	1.15 <i>m</i> , 1.64 <i>m</i>	42.0
4'				33.2		33.3		– ^f
5'	3.43 <i>s</i>	3.42 <i>s</i>		56.1	0.80 <i>m</i>	56.0	0.80 <i>m</i>	56.1
6'				20.4	1.56 <i>m</i>	20.5	1.56 <i>m</i>	– ^f
7'	5.78 <i>m</i>	5.72 <i>m</i>	2.02 <i>d</i> (12.0)	38.0	1.40 <i>m</i> , 1.52 <i>m</i>	42.1	1.38 <i>m</i>	42.0
8'	2.12 <i>brs</i>	2.10 <i>m</i>		44.5		44.9		– ^f
9'	1.36 <i>m</i>	1.44 <i>m</i>		56.5	1.06 <i>brd</i> (7.5)	56.7	1.07 <i>d</i> (7.4)	56.6
10'				39.4		39.4		– ^f
11'	1.95 <i>brd</i> (15.0) 2.33 <i>dd</i> (15.0, 8.8)	2.10 <i>m</i> 2.32 <i>dd</i> (14.2, 6.4)		18.5	1.62 <i>m</i>	18.3	1.62 <i>m</i>	– ^f
12'	5.17 <i>q</i> (6.5)	5.10 <i>q</i> (6.4)		26.2	1.62 <i>m</i>	26.3	1.62 <i>m</i>	27.1
13'	1.38 <i>d</i> (6.4)	1.35 <i>d</i> (6.4)	2.63 <i>brs</i>	43.4	2.12 <i>brs</i>	46.3	2.13 <i>brs</i>	46.5
14'	9.64 <i>s</i>	9.67 <i>s</i>		40.3	1.66 <i>m</i> , 1.98 <i>d</i> (11.5)	37.2	1.66 <i>m</i> , 1.99 <i>brd</i> (11.7)	37.5
15'	0.66 <i>s</i>	0.72 <i>s</i>	1.78 <i>d</i> (16.0) 1.91 <i>d</i> (16.0)	50.9	1.54 <i>brd</i> (14.5) 1.72 <i>dd</i> (14.5, 1.8)	53.3	1.54 <i>m</i> 1.72 <i>m</i>	53.4
16'				98.1		80.1		80.0
17'			3.99 <i>dd</i> (11.5, 4.0) 4.33 <i>dd</i> (11.5, 4.0)	63.5	4.50 <i>d</i> (11.5) 4.54 <i>d</i> (11.5)	69.6	4.51 <i>d</i> (11.5) 4.54 <i>d</i> (11.5)	70.2
18'			0.78 <i>s</i>	33.5	0.87 <i>s</i>	33.6	0.87 <i>s</i>	33.6
19'			0.74 <i>s</i>	21.5	0.82 <i>s</i>	21.5	0.82 <i>s</i>	22.0
20'			0.97 <i>s</i>	17.1	1.04 <i>s</i>	17.8	1.04 <i>s</i>	18.0

[#] OH: 3.25 *brt* (4.0)

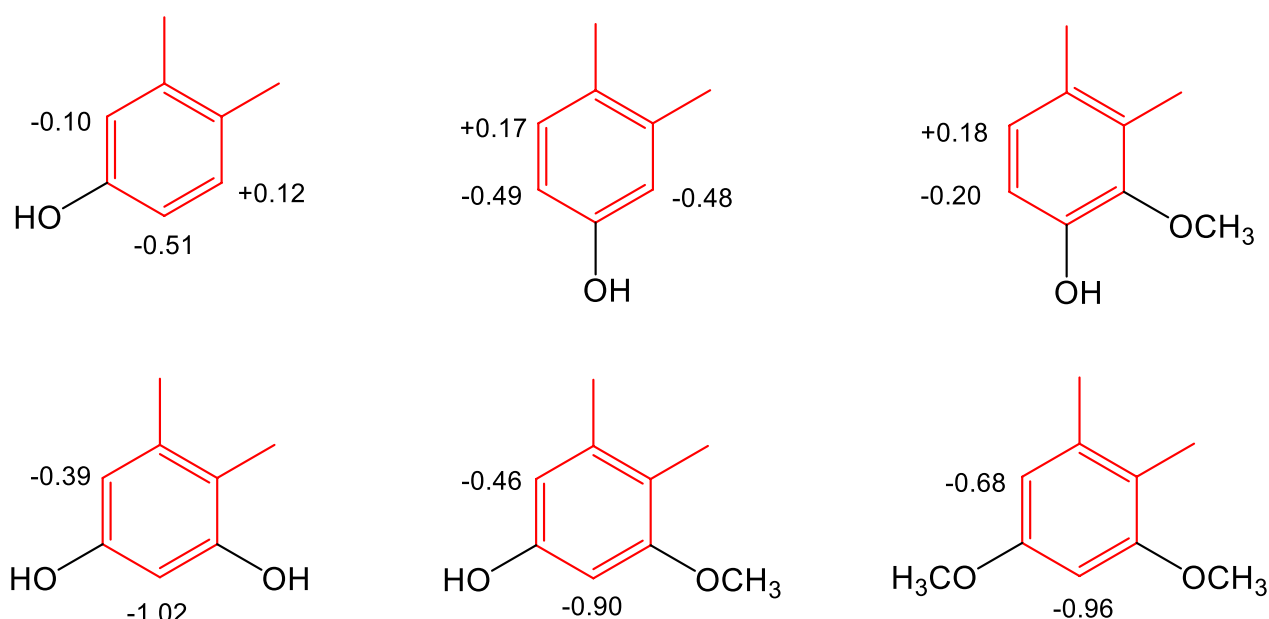


Fig. 4 Substituent effects on C-ring of 3,4-methylenedioxyaristolochic acids

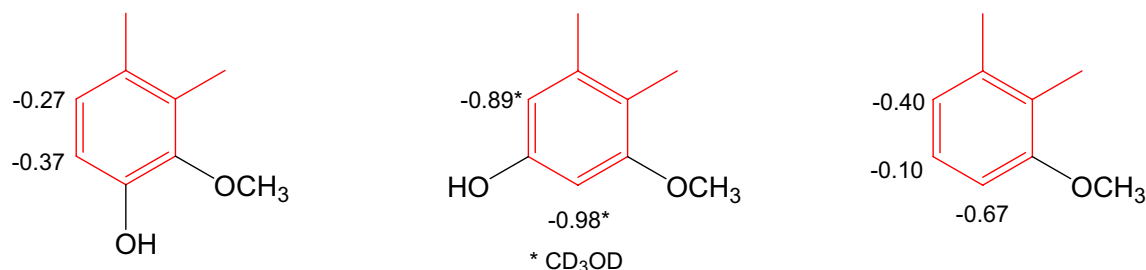


Fig. 5 Substituent effects on C-ring of 3,4-methylenedioxyaristolochic acids

analytical and preparative separation of AAs in *Aristolochia* herbs appears for the first time in the current review.

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Author contributions

PMG designed the study, wrote, and edited of the manuscript. DNP edited parts of the figures and tables. NNV, VTL, NDT, TPL prepared the references. TTTT read and edited the manuscript. All authors approved the final version of the manuscript.

Data availability

Not applicable.

Declarations

Competing interests

The authors declare no competing interests.

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