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An Intramolecular C_{Ar}–H...O=C Hydrogen Bond and the Configuration of Rotenoids*

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Abstract

Over the past half a century, the structure and configuration of the rotenoids, a group of natural products showing multiple promising bioactivities, have been established by interpretation of their NMR and electronic circular dichroism spectra and confirmed by analysis of single-crystal X-ray diffraction data. The chemical shift of the H-6' ¹H NMR resonance has been found to be an indicator of either a *cis* or *trans* C/D ring system. In the present study, four structures representing the central rings of a *cis*-, a *trans*-, a dehydro-, and an oxadehydro-rotenoid have been plotted using the *Mercury* program based on X-ray crystal structures reported previously, with the conformations of the C/D ring system, the local bond lengths or interatomic distances, hydrogen bond angles, and the H-6' chemical shift of these compounds presented. It is shown for the first time that a *trans*-fused C/D ring system of rotenoids is preferred for the formation of a potential intramolecular C₆–H₆'...O=C₄ H-bond, and that such H-bonding results in the ¹H NMR resonance for H-6' being shifted downfield.

Keywords

Millettia caerulea; Fabaceae; rotenoids; intramolecular C_{Ar}–H...O=C H-bond; NMR spectra and electronic effects; Conformation and configuration

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*Dedicated to Professor Dr. Max Wichtl in recognition of his outstanding contributions to natural product research

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Supporting information

Structures of rotenone, deguelin, and tephrosin and synthesis of deguelin; synthesis of [11-³H]-7-demethylmunduserone from 7,2'-di-hydroxy-4',5'-di-methoxyisoflavone; chemical and crystal structures and ECD spectrum of (–)-rotenone; structures of (+)-3-hydroxy-α-toxicarol and (–)-caeruleanone D and their ECD spectra; structures and molecular models of (–)-rotenone, (–)-deguelin, (–)-tephrosin, (2*R*,3*R*,1"*R*,2"*S*)-1",2"-dihydro-1",2"-dihydroxytephrosin, and (+)-usararotenoid A, and their epimers; structures and molecular models of (–)-13-homo-13-oxa-2,3-dehydrorotenone and 2,3-dehydrosermundone; structures and selected δ values for several *cis*-rotenoids and their *trans* isomers.

Conflict of Interest

The authors declare no competing financial interest.

Introduction

Rotenoid natural products, occurring mainly in species of the plant family Fabaceae, are the major active components of several botanical insecticides, miticides, and piscicides, including derris (the dried roots of *Derris elliptica* Benth.), cubé (the dried roots of *Lonchocarpus utilis* A. C. Smith), timbo (*Lonchocarpus urucu* Killip & A. C. Smith.), and barbasco (*Lonchocarpus urucu* Killip & A. C. Smith) [1]. These natural products have been characterized as a group of biogenetically advanced isoflavonoids [2, 3], with rotenone being a primary member that occurs in dried derris roots at concentration levels up to 12–15% [1]. Other predominant representatives are deguelin and tephrosin, of which tephrosin has been proposed as being an oxidized product of deguelin [1], a compound synthesized from rotenone and tephrosin [4, 5].

Over the past 100 years, rotenoids have been used as insecticides or piscicides to contribute to global farming [1], owing to their inhibitory activity against reduced nicotinamide adenine dinucleotide (NADH):ubiquinone oxidoreductase (Complex I) [6, 7]. Beyond this property, rotenoids have shown antibacterial (against *Helicobacter pylori*) [8], antitumor [9], antiinflammatory [10], and antileishmanial and antimalarial activities [11, 12]. The antitumor propensity of rotenoids, which was reported to correlate with their Complex I inhibitory effects, has attracted an increasing interest [13–15]. In our previous work, (–)-rotenone and its analogues and (–)-tephrosin were found to exhibit cytotoxicity toward a small panel of human cancer cell lines [16–19], with similar effects observed for deguelin and 13-homo-13-oxa-2,3-dehydrodeguelin by other groups [20, 21]. Interestingly, deguelin was found to show *in vivo* cancer chemopreventive and antitumor effects [22, 23]. Tumor growth was inhibited, when thirty six-week-old male athymic BALB/c nu/nu mice bearing H460 human lung tumor xenografts were treated with deguelin (1 or 4 mg/kg, once every three days for 30 days) [23].

Their potent and promising bioactivities have triggered a wide interest in the search of new rotenoid analogues [24], and the need for their correct structural determinations has proved to be critically important for the further development of these agents. The structure of the parent compound, rotenone, has been ascertained by investigation of its NMR spectroscopic data [25, 26] and confirmed by X-ray crystallography [27], with its (2*S*, 3*S*, 2''*R*) absolute configuration established by a series of chemical reactions [28] and confirmed by single-crystal X-ray diffraction analysis for 5'-bromorotenone [29]. The electronic circular dichroism (ECD) spectra of rotenone and several analogues have been investigated, with the Cotton effects (CEs) characterized [19, 30]. Although the CEs above 300 nm were not suggested as reliable configurational assignments for some 3-substituted rotenoids [31], it has been well documented that these CEs are suitable for indicating the absolute configuration at C-2 and C-3 of 3-hydroxyrotenoids [19, 32].

Two different fusions, the thermodynamically favored *cis*-fused and the unfavored *trans*-fused C/D ring systems, occur in rotenoids [32], and a *trans*-C/D ring may be changed to its *cis* form by chemical synthesis [33]. It has been proved challenging on occasion to identify these different fusion modes, owing to the overlapped NMR resonances observed for their H-2 and H-3 protons and any methoxy groups present. Fortunately, the chemical shift value

for H-6' of rotenoids was found to be strongly deshielded for a *trans*-C/D ring junction, when compared with this resonance for rotenoids containing a *cis*-C/D ring system [32, 33]. Accordingly, this value has been utilized as an effective indicator of the C/D fusion mode for rotenoids. Previous explanations for these observations have focused mainly on conformational and anisotropic effects from the C/D ring system [25, 26, 33]. In the present study, an alternative rationale for the H-6' chemical shift in serving as an indicator of the relative configuration of rotenoids has been presented, with an intramolecular C_Ar-H•••O=C hydrogen bond between H-6' and oxygen of the C-4 carbonyl group (C₆'-H₆'•••O=C₄ H-bonding) being conjectured.

Results and Discussion

Based on their structural characteristics, naturally occurring rotenoids can be classified into six major sub-groups, comprising *cis*-rotenoids (with a *cis*-fused C/D ring), *trans*-rotenoids (with a *trans*-fused C/D ring), 2,3-di-*epi-cis*-rotenoids (with a reversal of the C-2 and C-3 positions when compared with the *cis*-rotenoids), dehydrorotenoids (with a 2,3-double bond), oxarotenoids (with an additional oxygen inserted between C-3 and C-1' of a *cis*- or a *trans*-rotenoid), and oxadehydrorotenoids (with an additional oxygen inserted between C-3 and C-1' of a dehydrorotenoid) (Fig. 1). In turn, most of these sub-groups of rotenoids can be divided further into two groups, “rotenone-like” rotenoids, containing a C-7 furan-fused 2-methyl-3-*O*-1-buten-4-yl unit, and “deguelin-like” rotenoids, incorporating a C-7 pyran-fused 3-methyl-3-*O*-1-buten-1-yl unit, respectively [19] (Fig. S1, Supporting Information). The “deguelin-like” rotenoids might be derived from “rotenone-like” rotenoids, as evidenced by successful synthesis of deguelin from rotenone and tephrosin [4, 5] (Fig. S1, Supporting Information) and the co-occurrence of both types of rotenoids in some plant species [13–19].

Rotenoids have been postulated as being a group of biogenetically advanced isoflavonoids, with an additional ring D formed between C-2 and the C-2' methoxy group, as evidenced by biosynthesis of a rotenoid from an isoflavonoid precursor in plants and the chemical synthesis of (±)-[11-³H]-7-demethylmunduserone from 7,2'-dihydroxy-4',5'-dimethoxyisoflavone (Fig. S2, Supporting Information) [2, 3]. Such a hypothesis was also supported by the co-occurrence of rotenoids and isoflavonoids in some plants [19, 20]. Following their biogenetic origin, rotenoids can be numbered structurally following the same system as that used for isoflavonoids but different from the rotoxenoids (Fig. 1).

The bent structure of rotenone with a (2*S*, 3*S*, 2''*R*) absolute configuration has been determined unequivocally by analysis of its single-crystal X-ray diffraction data [27, 29], and its ECD spectrum showing the pronounced negative CEs around 209 and 276 nm and a positive CE at 237 nm, along with relatively weak negative CE around 307 nm and positive CEs at 333 and 352 nm, has been reported (Fig. S3, Supporting Information) [19, 30]. These ECD characteristics have been proved useful in a determination of the absolute configuration of other “rotenone-like” rotenoids, and, as mentioned above, the CEs above 300 nm have been suggested as a reliable reference to indicate the absolute configuration at C-2 and C-3 of 3-hydroxyrotenoids [19, 32], as exemplified by the ECD spectra of (+)-3-hydroxy-α-toxicarol and (–)-caeruleanone D (Fig. S4, Supporting Information) [19].

Both *cis*- and *trans*-rotenoids have been characterized from higher plants, and the structures of some representatives have been confirmed by X-ray diffraction analysis [11, 15, 33]. Identification of the *cis*- or *trans*-fused C/D ring system is critically important in determining the absolute configuration of rotenoids, but overlapped NMR resonances for H-2, H-3, and any methoxy groups present may make this challenging. The ^1H NMR chemical shift value for H-6' of *cis*-rotenoids (in the range δ 6.0–7.1) and for this same proton of *trans*-rotenoids (δ 7.5–8.5) has been proved sufficiently diagnostic, and the rationale for this has been proposed as resulting from the anisotropic effects of the C-4 carbonyl group and the conformational effects of the C/D ring system, based on analysis of Dreiding molecular models and the NMR spectroscopic data of various rotenoids [25, 26, 33].

To evaluate the conformational effects from the C/D ring system, plots showing the conformation of the central ring system of (–)-(2*R*,3*R*,1"*R*,2"*S*)-1",2"-dihydro-1",2"-dihydroxytephrosin (**1**, a *cis*-rotenoid) and (+)-usararotenoid A (**2**, a *trans*-rotenoid) have been generated in the present work (Fig. 2). These were based on their reported X-ray crystal structures [11, 15], using a crystal structure visualization program, *Mercury* version 3.8 [34], with the X-ray coordinates obtained from CIF files deposited in the Cambridge Structural Database [35]. As shown in Fig. 2, the conformation of the C/D ring system of **1** was found to be obviously different from that of **2**, as supported by the different “torsion angles” for H5–C5•••C4–O4, C2'–C1'•••C4–O4, and H6'–C6'•••C4–O4 of both compounds. These “torsion angles” were found to be –2.4°, 168.4°, and –3.5°, respectively, for **1**, and –20.3°, –129.1°, and 46.2°, respectively, for **2** (Table 1). The *cis*-C/D ring system in **1** was bent in a “roof-tile”-like shape, but the *trans*-fused C/D ring in **2** was geometrically more planar than that *cis*-fused in **1** (Fig. 2). The similarity of these conformations has been observed in the molecular modeling of all (–)-rotenone and its epimers, (–)-deguelin and its epimers, (–)-tephrosin and its epimers, (2*R*,3*R*,1"*R*,2"*S*)-1",2"-dihydro-1",2"-dihydroxytephrosin and its epimers, and (+)-usararotenoid A and its epimers (Figs. S5–S9, Supporting Information).

To test the shielding effects from the C-4 carbonyl group, the interatomic distance between C-6' and oxygen of the C-4 carbonyl group (C6'•••O4) of **1** and **2** has been calculated, using the same *Mercury* program as mentioned above [34]. The value (3.169 Å) of **1** was found to be longer than that (2.940 Å) of **2** (Table 2). The chemical shift for H-6' of compounds **1** (δ 6.53 measured from CDCl_3) [15] and **2** (δ 8.22 measured from $\text{C}_5\text{D}_5\text{N}$) [33] (Fig. 2), along with the same tendency of a more downfield signal for H-6' of *trans*-rotenoids than that of *cis*-rotenoids observed in the different deuterated solvents employed [33], indicated that the H-6' proton of *trans*-rotenoids is more deshielded than that of *cis*-rotenoids. Consistent with this observation, chemical shift values for H-6' of (–)-13-homo-13-oxa-2,3-dehydrorotenone (**3**) (δ 6.84) and of (–)-2,3-dehydrorotenone (δ 8.46) were reported in our previous study [19]. Inspection of the ^1H NMR spectroscopic data of **3** and (–)-2,3-dehydrorotenone showed that an obviously different NMR resonance was observed only for the H-6' proton, with that for other protons found to be closely similar [19]. This indicates that an intramolecular H-bond between H-6' and the C-4 carbonyl group and not an anisotropic effect from the C-4 carbonyl group affects greatly the H-6' ^1H NMR resonance of certain rotenoids.

To test this hypothesis, plots showing the conformation of the C/D ring system of (–)-13-homo-13-oxa-2,3-dehydrorotenone (**3**, an oxadehydrorotenoid) and 2,3-dehydrosermundone (**4**, a dehydrorotenoid) were drawn (Fig. 3), based on their X-ray crystal structures reported previously [36, 37], using the same procedure as mentioned above for compounds **1** and **2**. A similar conformation with an almost planar C/D ring system was found for both **3** and **4**, but the C6'...O4 and O4...H6' interatomic distances (4.405 and 4.005 Å, respectively) observed for **3** were found to be much longer than those (2.881 and 2.250 Å, respectively) calculated for **4** (Table 2 and Fig. 3). In turn, different chemical shifts for H-6' of **3** (δ 6.94) [36] and **4** (δ 8.24) [38] have been reported. This evidence strongly supports the formation of a potential intramolecular C₆–H₆...O=C₄ H-bond. The conformation of the C/D ring series of **3** and **4** has also been confirmed by their molecular modeling (Figs. S10 and S11, Supporting Information).

Conformational effects on the NMR resonances observed for rotenoid compounds have been discussed [39], but an intramolecular C₆–H₆...O=C₄ H-bond has not been assumed for such an issue by any previous studies. The existence of non-classical C–H...O H-bonds was confirmed by analysis of the total charge density method [40], and an intramolecular N–H...O=C H-bond between the H atom of a *cis* thioamide and a carbonyl oxygen atom has been supported by single-crystal X-ray diffraction analysis, with a *trans-cis* geometry of the almost planar thiourea unit found stabilized by this H-bonding [41]. Recently, an intramolecular C_{Ar}–H...O=C H-bond has been reported, based on X-ray diffraction data [42], and the chemical shift of an intramolecular “non-classical” H-bonding C–H proton was found shifted downfield about 1 ppm [43]. This downfield chemical shift has been proposed as a potential means of evaluating the H-bond, and the “short” C...O (<3.3 Å) and O...H (<2.6 Å) interatomic distance and the favored orientation have been evidenced as consequence of such H-bonding [43].

A planar-like C/D ring system was shown in the *trans*-rotenoid **2** (Fig. 2), which provided a potential pseudo six-membered ring over the bonds O4–C4–C3–C1'–C6'–H6'...O4, as indicated by a hydrogen bond angle 107.6° for C6'–H6'...O4 (>90°) [42]. This conformation and the pseudo six-membered ring would be favored for an intramolecular C_{Ar}–H...O=C H-bond between H-6' and O-4, as indicated by the “short” C6'...O4 [2.940 Å (<3.3 Å)] and O4...H6' [2.492 Å (<2.6 Å)] interatomic distances (Fig. 2 and Table 2) [43]. Such an intramolecular C₆–H₆...O=C₄ H-bond would lead to a strongly deshielded ¹H NMR resonance at δ 8.22 for H-6' of **2** [33], when compared with that at δ 6.53 for the same proton of **1** [15]. However, the planar-like conformation of the C/D ring system is not apparent in the *cis*-rotenoid **1**, and the non-planar C/D ring system is unfavored for the formation of an intramolecular C₆–H₆...O=C₄ H-bond, as implied by its “long” C6'...O4 (3.169 Å) and O4...H6' (2.601 Å) interatomic distances (longer than those of **2**) (Fig. 2 and Table 2) [43].

Such a proposed intramolecular C₆–H₆...O=C₄ H-bond is supported by the plots drawn for compounds **3** and **4**. As shown in Fig. 3, although a planar-like C/D ring system is evidenced for **3** and **4**, the “long” C6'...O4 [4.405 Å (>3.3 Å)] and O4...H6' [4.242 Å (>2.6 Å)] [43] interatomic distances observed in **3** prohibit the formation of intramolecular C₆–H₆...O=C₄ H-bond. Thus, a normal aromatic ¹H NMR resonance at δ 6.93 for H-6' is observed for **3**

[36]. Differentially, the “short” C6'•••O4 [2.881 Å (<3.3 Å)] and O4•••H6' [2.250 Å (<2.6 Å)] [43] interatomic distances of **4** supported an intramolecular C₆–H₆•••O=C₄ H-bond, as indicated by the downfield signal at δ 8.24 for H-6' of **4** (Fig. 3) [38].

As summarized in Fig. S12 (Supporting Information), the downfield signal observed for the ¹H NMR resonance for H-6' of *trans*-rotenoids is not influenced by the choice of solvents used, as supported by the data measured in the same or different deuterated solvents for several rotenoids and their epimers [15, 18, 19, 33, 36, 44, 45]. Comparison of the ¹H NMR chemical shift values for H-5, H-6, H-3', and H-6' or other protons of **3** and (–)-2,3-dehydrorotenone showed that no obvious changes were observed for any protons other than H-6', and a similar observation was evidenced in other rotenoids (Fig. S12, Supporting Information) [15, 18, 19, 33, 36, 44, 45]. This indicates that an anisotropic effect from the C-4 carbonyl group is not a major contributor to the downfield signal for the ¹H NMR resonance for H-6' of *trans*-rotenoids.

Recently, an intramolecular H-bond between the hydroxy group at C-5 (OH-5) and the C-4 carbonyl group (C₄=O) of flavonoids and isoflavonoids has been reported, as indicated by a downfield signal around δ 12.5 for OH-5, when compared with those observed in the range δ 5.0–10.0 assigned for other aromatic hydroxy groups [46]. Also, a non-classical intramolecular C_{Ar}–H•••O=C H-bond has been demonstrated for some (diaryl)tetrahydrofuranones by their X-ray diffraction data [42]. Inspection of the partial structural units of C₅–O–H•••O=C₄ of flavonoids, isoflavonoids, or rotenoids and that for the H-bond of (diaryl)tetrahydrofuranones shows that these structural units are closely comparable, indicating a potential intramolecular C₅–H₅•••O=C₄ H-bond for rotenoids and another C₆–H₆•••O=C₄ H-bonds for *trans*-rotenoids. Such an assumption has been supported by the O4•••H6' interatomic distances, C6'–H6'•••O4 hydrogen bond angles, and the chemical shift values for H-5 and H-6' ¹H NMR resonances of *trans*-rotenoids (Table 2 and Figs. 2 and 3 and Fig. S12, Supporting Information), which are consistent with those calculated for other H-bonds [42, 43, 47].

The observations summarized herein are a consequence of the different conformations that occur in *cis*- and *trans*-rotenoids. The conformation of the *trans*-rotenoids is favored for the formation of an intramolecular C₆–H₆•••O=C₄ bent H-bond, which leads a strongly deshielded ¹H NMR resonance around δ 8.0 for their H-6' proton, with this resonance value used to differentiate the *trans*-rotenoids from their *cis* isomers.

Rotenoid natural products contain diverse structures and show multiple promising bioactivities. The correct determination of the conformation and configuration of these natural products plays a vital role for the further development of these compounds. The ¹H NMR chemical shift of H-6' occurring in an unsubstituted manner in natural rotenoids has been used to indicate the presence of a *cis*- or a *trans*-rotenoid, and this is proposed to be due to an intramolecular C₆–H₆•••O=C₄ bent H-bond. Moreover, this type of configuration determination involving intramolecular H-bond formation may be evident in other natural product structural classes.

Materials and Methods

Plant material and extraction and isolation

A fruit sample of *Millettia caerulea* (Graham) Baker (Fabaceae-Papilionoideae) was collected in Vietnam, as reported in our previous publication [19]. The rotenoids (–)-rotenone, (+)-3-hydroxy- α -toxicarol, and (–)-caeruleanone D were isolated from a methanol extract of the milled air-dried fruits of *M. caerulea* and determined structurally by analysis of their spectroscopic data [19].

Plots of crystal structure

The plots of various X-ray structures were drawn using the *Mercury* program, version 3.8, of the Cambridge Crystallographic Data Centre (CCDC, Cambridge) [34], with bond lengths and angles and torsion angles calculated with this same program. The X-ray coordinates were obtained from the CIF files deposited in the Cambridge Structural Database CSD) [35].

3D Structural modeling

The 3D structural modeling showing the conformation of the central ring system of rotenoids was obtained using the PerkinElmer ChemBio3D Ultra 14.0 suites, with the stick mode displayed and molecule translated and rotated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Structures of the rotoxin, isoflavonoid, and rotenoid skeletons.

Fig. 2.

Structures of (-)-(2*R*,3*R*,1''*R*,2''*S*)-1'',2''-dihydro-1'',2''-dihydroxytephrosin (**1**) [(A)] and (+)-usararotenoid A (**2**) [(C)] and their partial crystal structures showing the conformation of the central ring system [(B) and (D)], which were generated by *Mercury*, version 3.8 [34], and based on reported X-ray crystallographic data [11, 15]. Chemical shift (δ) values were measured in CDCl₃ for **1** [15] and in C₅D₅N for **2** [33], with the interatomic distances expressed in angstroms. A potential intramolecular C₆-H₆...O=C₄ H-bond may be formed in a *trans* rotenoid (**2**), which showed a planar-like conformation, but not in a *cis* rotenoid (**1**), which has a bent structure conformation.

Fig. 3.

Structures of (-)-13-homo-13-oxa-2,3-dehydrorotenone (**3**) [(A)] and 2,3-dehydrosermundone (**4**) [(C)] and their partial crystal structures showing the conformation of the central ring system [(B) and (D)], which were generated by *Mercury*, version 3.8 [34], and based on reported X-ray crystallographic data [36, 37]. Chemical shift (δ) values were measured in CHCl₃ for both compounds [36, 38], with the interatomic distances expressed in angstroms. A potential intramolecular C₆–H₆•••O=C₄ H-bond may be formed in **4** but not in **3**, owing to a “short” (2.250 Å) H₆•••O interatomic distance in **4**, which is possible for the formation of an intramolecular H-bond.

Table 1

“Torsion angles” of compounds **1** and **2**^a.

Compound	H5–C5···C4–O4	C2'–C1'···C4–O4	H6'–C6'···C4–O4
1	–2.4	168.4	–3.5
2	–20.3	–129.1	46.2

^a“Torsion angles” expressed in degrees refers the angles between two bonds bonded to a third atom or bond.

Table 2Selected bond lengths, interatomic distances, and hydrogen bond angles for compounds **1–4**.

Compound	C4–O4 ^a	C6'...O4 ^b	C6'–H6'...O4 ^c
1	1.235	3.169	118.8
2	1.216	2.940	107.6
3	1.235	4.405	108.9
4	1.269	2.881	124.5

^aBond lengths expressed in angstroms (Å);^bInteratomic distances expressed in angstroms (Å);^cHydrogen bond angles expressed in degrees.