New Rotenoids from the Root Bark of Jamaican Dogwood (*Piscidia erythrina* L.)

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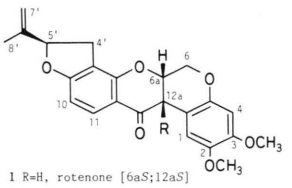
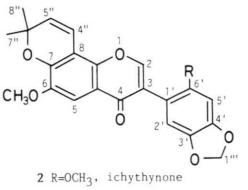
A further investigation of the root bark constituents of Jamaican dogwood *(Piscidia erythrina)* has revealed two new pyrano-rotenoids, (+)-erythynone and (+)-12a-hydroxyerythynone. Both compounds co-occur with (−)-rotenone, and two additional rotenoids [(−)-12a-hydroxyrotenone and (−)-villosinol] not previously isolated from *P. erythrina.* Root extracts were also found to contain the rare isoflavone durmillone. The stereochemistry of all five *Piscidia* rotenoids was examined by ORD and CD spectrometry. These studies indicated that erythynone and 12a-hydroxyerythynone were antipodal to naturally occurring (−)-6a*S*;12a*S*-rotenone at the B/C ring junction.

Introduction

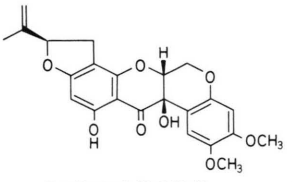
Root bark of the Jamaican dogwood [*Piscidia erythrina* L. = *P. piscipula* (L.) Sarg.; Leguminosae-Papilionoideae] has long been of interest because of its fish-poisoning properties, a feature at least partly attributable to the presence of two isoflavonoids, the rotenoid rotenone (**1**) and the isoflavone ichthynone (**2**) [1, 2]. As reported in our earlier paper [3], *P. erythrina* root bark collected in the Yucatan region of Mexico contains large quantities of piscidone, piscerythrone and 6′-prenylpiscerythrone (all isoflavones) [2, 4, 5], in addition to **1** and **2,** and various minor isoflavonoids. However, we were unable to detect the previously reported isoflavone jamaicin [6] or any known *Piscidia* rotenoids (*e.g.* millettone, isomillettone or dehydromillettone) [2, 5] except for rotenone itself. These differences may reflect the existence of chemical races within the species *P. erythrina* [5] since all the studies apart from our own appear to have been carried out on plant material of non-Mexican (*e.g.* Jamaican [2]) origin.



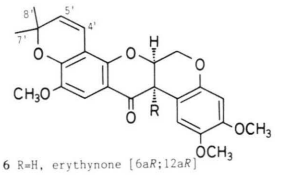
1 R=H, rotenone [6a*S*;12a*S*]
4 R=OH, 12a-hydroxyrotenone [6a*R*;12a*R*]



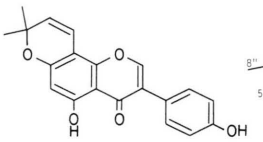
2 R=OCH_3, ichythynone
3 R=H, durmillone



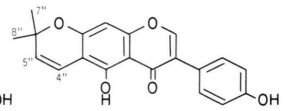
5 villosinol [6a*R*;12a*R*]



6 R=H, erythynone [6a*R*;12a*R*]
7 R=OH, 12a-hydroxyerythynone [6a*S*;12a*S*]



8 derrone



9 alpinumisoflavone

We have now undertaken a further survey of the minor isoflavonoids in *P. erythrina* root bark. This study has revealed another isoflavone (durmillone (**3**)) [7, 8] and two 12a-hydroxyrotenoids (12a-hydroxyrotenone or rotenolone (**4**), and villosinol (**5**)) previously obtained from other legumes [9]. Additionally, however, the *Piscidia* extracts contained two new rotenoids, erythynone (**6**) and 12a-hydroxyerythynone (**7**), each with a D-ring substituted as in the equivalent ring A of ichthynone (**2**) and durmillone (**3**). Rotenoids **6** and **7** were found to be dextrorotatory, in contrast to rotenone, 12a-hydroxyrotenone and villosinol which were laevorotatory.

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Results and Discussion

A methanolic extract of *P. erythrina* root bark was fractionated and purified by column and thin-layer chromatography (see ref. [3] and the Experimental section) to yield the isoflavone durmillone (**3**), and the rotenoids 12a-hydroxyrotenone (**4**), villosinol (**5** = 11,12a-dihydroxyrotenone), erythynone (**6**) and 12a-hydroxyerythynone (**7**). Several of the isoflavonoids (*e.g.* rotenone (**1**), ichthynone (**2**), piscidone and piscerythrone) described in our earlier paper [3] were also re-isolated. Erythynone and its 12a-hydroxy derivative are described here for the first time. Although six rotenoids (rotenone (**1**), millettone, isomillettone, dehydromillettone, sumatrol and deguelin) are known to occur in *P. erythrina* root bark [2, 5], the presence of 12a-hydroxyrotenoids (rotenolones [10]) does not appear to have been reported.

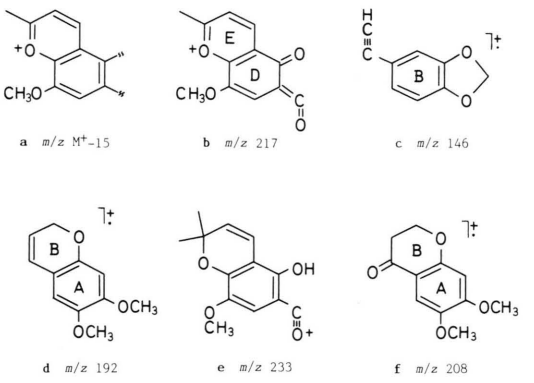

Fig. 1. Mass fragments mentioned in the text.

Cis-5′*R*;6a*S*;12a*S*-rotenone (**1**) was identified by direct comparison (UV, MS, ^{1}H NMR, CD and silica gel TLC) with an authentic sample. *Piscidia*-derived rotenone was strongly laevorotatory, $[\alpha]_D^{23}$ − 148° (MeOH).

The identification of 12a-hydroxyrotenone (**4**) was based on a comparison of UV, MS and ^{1}H NMR data with published literature values [11]. *Cis*-orientation at the B/C ring junction was evident from the comparatively high-field 1-H signal at δ 6.64 (*cf.* *trans*-12a-hydroxyrotenones where 1-H appears at about δ 8.0 [11], whilst the stereochemistry at C-5′ (ring E) was deduced to be *R*, as in natural rotenone, from the negative Cotton effect (CD spectrum) given at 472 nm by the osmate ester-pyridine complex [12, 13]. Thus, only the absolute stereochemistry at C-6a/12a remained to be determined.

According to Unai *et al.* [14], strongly laevorotatory 12a-hydroxyrotenoids ($[\alpha]_D$ − 170° to − 190°) are 6a*R*;12a*R* whereas dextrorotatory compounds, or those giving a very small laevorotatory value ($[\alpha]_D$ − 3° to + 31°) are 6a*S*;12a*S* (*cf.* data for the 12a-hydroxyrotenones denoted Ax/Dx and Ay/Dy in ref. [14]). Since 12a-hydroxyrotenone from *P. erythrina* was found to have a high negative optical rotation ($[\alpha]_D^{23}$ − 115°), the 6a*R*;12a*R* absolute configuration must apply*. A positive first Cotton (CD) effect at 359 nm, similarly given by **1**, also supported the 6a*R*;12a*R* stereochemistry of **4** [14]. 12a-Hydroxyrotenone has previously been found in seeds of *Piscidia mollis* [9], and in the roots and other parts of various species from related genera (*e.g.* *Derris*, *Lonchocarpus* and *Tephrosia* [9]).

Apart from **4**, the root bark of *P. erythrina* also contained villosinol (**5**), a 12a-hydroxyrotenoid already known to occur in pods of *Tephrosia villosa* [15]. As authentic villosinol was not available for comparative purposes, identification of the *Piscidia*-derived material was based, like that of **4**, on data from spectroscopic studies. Substitution of rings A and B as shown for **5** (M^+ 426, $C_{23}H_{22}O_8$) was evident from both the intense mass fragment at *m/z* 208 (**f**) indicative of a 2,3-dimethoxy-12a-hydroxyrotenoid (*cf.* data for **4**) [8], and the ^{1}H NMR spectrum which afforded A/B-ring signals superimposable on those of 12a-hydroxyrotenone (**4**). Hydroxylation at C-11 was confirmed by the 22 nm bathochromic shift of the UV (MeOH) maximum at 299 nm upon addition of $AlCl_3$ [16].

In order to establish that the D/E-ring junction was angular (**C**) rather than linear (**D**), the ^{1}H NMR spectrum of **5** was measured in pyridine-d_5 and $CDCl_3$. The solvent effect [17] on the single

* Although introduction of a 12a-hydroxyl group into a (−)-6a*S*[H];12a*S*[H] rotenoid (*e.g.* rotenone) has no effect on the sign of rotation, in accordance with chirality rules the stereochemistry becomes *R* because of a change in priority of the substituents at C-6a and C-12a. In the case of a dextrorotatory 6a*R*[H];12a*R*[H] rotenoid, hydroxylation at C-12a changes the stereochemistry to *S*. A comparable situation has been previously noted in the pterocarpan/6a-hydroxypterocarpan series of isoflavonoid compounds [18].

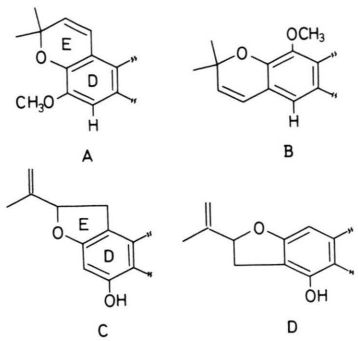

Fig. 2. Part structures showing angular (**A** and **C**) or linear (**B** and **D**) substitution.

A-ring proton (6-H; Δδ pyridine-d_5→$CDCl_3$ = 0.257 ppm) was then compared with shifts given under identical conditions by 6-H and 8-H respectively of the model pyranoisoflavones derrone (**8,** angular-type, 6-H; Δδ = 0.260 ppm) and alpinumisoflavone (**9,** linear-type, 8-H; Δδ = 0.167). The shift value for 6-H of **5** was almost identical with that of **8** indicating an angular D/E ring fusion (**C**). Although the solvent effect (Δδ) for an aromatic proton *ortho* to an OH group is often 0.4–0.5 ppm [17], the weak interaction between a pyridine molecule and the H-bonded hydroxyl of **5** presumably accounts for the rather small Δδ values obtained for 6-H of **5** and **8.** Support for part structure **C** was also provided by the failure of **4** to give a blue Gibbs test colour [19, 20]. The stereochemistry at C-5′ of **5** was found to be *R* from the negative Cotton effect given by the osmate ester-pyridine complex at 475 nm [12, 13]. Moreover, since **5** was laevorotatory ($[\alpha]_D^{23} - 38°$) and gave a positive first Cotton effect (CD spectrum) at 348 nm, the B/C ring absolute stereochemistry must be 6a*R*;12a*R* [14]. *Cis*-fusion of the B/C rings was indicated by the ^{1}H NMR signal at δ 6.70 ($CDCl_3$ spectrum) [10].

In addition to compounds **1, 4** and **5,** the *Piscidia* extracts also contained two closely related rotenoids (erythynone **6,** and 12a-hydroxyerythynone **7**) not previously found as natural products. Apart from a *cis*-fused B/C ring system (1-H at δ 6.76 [10]) the ^{1}H NMR spectrum of **6** (M^+ 424, $C_{24}H_{24}O_7$) clearly indicated that rings A and B were substituted as in rotenone (**1**). The MS base peak fragment at *m/z* 192 (**d**) was also typical of a rotenoid with A-ring (C-2/3) dimethoxylation [21]. Other ^{1}H NMR signals at δ 1.37, 1.47 (both 3H, two s, 2 × CH_3), 5.74 and 6.85 (both 1H, two d, with J = 9.9 Hz, olefinic protons) were attributable to a 2,2-dimethylpyrano side structure which was assigned (8→9[O]) to ring D, with the remaining proton (δ 7.24s) and $OC\underline{H}_3$ group (δ 3.80) being placed at C-11 and C-10 respectively by analogy with ichthynone (**2**). Irradiation at δ 3.80 (C-10 $OC\underline{H}_3$) enhanced the signal intensity of the C-11 proton (NOE effect) confirming part structure **A,** rather than **B,** for ring D of erythynone. MS fragments observed at *m/z* 409 ($M^+ - 15$, 14%), 233 (19%) and 217 (8%) can thus be represented as **a, e** and **b** respectively. As erythynone was dextrorotatory ($[\alpha]_D^{23} + 25°$) and gave a negative first Cotton effect at 370 nm opposite to that of (−)-rotenone, the absolute configuration at C-6a and C-12a must be both *R* [14].

The final rotenoid (**7**) gave M^+ 440 ($C_{24}H_{24}O_8$ = 6 + [O]) with a base peak fragment at *m/z* 208 (**f**) indicative of a 2,3-dimethoxy-12a-hydroxyrotenoid (*cf.* MS data for **4** and **5**). Fragments at *m/z* 233 (**e,** 18%) and 217 (**b,** 7%) were attributed to a D-ring substituted as in erythynone (**6**). ^{1}H NMR data for rings A/B/C and D/E (side structure) closely resembled those given by 12a-hydroxyrotenone (A/B/C-rings) and erythynone (D/E-rings) respectively, and additionally confirmed the *cis* nature of the B/C ring junction (1-H at δ 6.68 [10]). Like erythynone, the 12a-hydroxy derivative (**7**) was dextrorotatory ($[\alpha]_D^{23} + 28°$) and gave a similar CD curve (negative first Cotton effect at 370 nm) allowing the absolute stereochemistry to be defined as 6a*S*;12a*S* (see footnote).

Piscidia root bark is rich in isoflavones [2–5] and apart from known compounds the present study yielded durmillone (**3**), the 2′-deoxy analogue of ichthynone (**2**). The ^{1}H NMR spectrum of **3** closely resembled that of ichthynone, affording signals characteristic of a 2,2-dimethylpyrano substituent, and an aromatic singlet (δ 7.48) attributable to 5-H deshielded by the carbonyl group (*cf.* 5-H of **2** at δ 7.44). The isoflavone 2-H signal appeared at δ 8.26 [22]. Three aromatic *m-, o-* and *o/m*-coupled protons (2′-, 5′- and 6′-H) were assigned to ring B together with an $O-C\underline{H}_2-O$ group (δ 6.04s, *cf.* **2,** δ 6.00s) which can only be located at C-3′/4′ if 4′-oxygenation is assumed. Apart from the base peak fragment at *m/z* 363 ($M^+ - 15$, **a**), the MS of *Piscidia* durmillone (M^+ 378, $C_{22}H_{18}O_6$) gave signals at *m/z* 217 (RDA frag-

ment from ring A as given by ichthynone, **b,** 3%) and *m/z* 146 (RDA fragment from ring B with a methylenedioxy substituent, **c,** 3%). Durmillone has previously been obtained from species of *Millettia (e.g. M. ferruginea)* [7–9], a genus closely allied to *Piscidia.*

Experimental

General procedures

Analytical and preparative thin-layer separations were carried out on Merck pre-coated silica gel plates (F-254; layer thickness, 0.25 or 0.5 mm) using the following solvent systems: (a) CM = $CHCl_3$–MeOH, (b) HE = *n*-hexane–EtOAc, (c) CAAm = $CHCl_3$–acetone–conc. NH_4OH, and (d) HEAm = *n*-hexane–EtOAc–conc. NH_4OH (upper layer). Details relating to the composition of solvent systems are given at the appropriate point(s) in the text. All compounds were eluted from chromatograms with EtOAc. Detection of isoflavones and rotenoids on developed thin-layer plates was by inspection under long (365 nm) and short (254 nm) wavelength UV light, and by the colours formed after spraying with Gibbs reagent [19, 20]. Instrumental analyses (UV, MS, ORD-CD and ^{1}H NMR) were undertaken using the equipment and conditions described in our earlier papers [13, 20]. The stereochemistry at C-5′ (E-ring) of isolated rotenoids was determined by CD measurements carried out on the osmate ester-pyridine complex [12, 13].

Extraction and purification of isoflavonoids

The root bark of *Piscidia erythrina* L. was extracted with MeOH, the extractives then being initially fractionated as reported in our earlier paper [3]. Rotenone used for comparative purposes had previously been isolated (2.5 g) from *P. erythrina* [3]. Silica gel column fraction Fr–9 (see ref. [3]), eluted with 15% EtOAc in benzene, was concentrated and chilled to yield precipitates (1st, 1628 mg; 2nd, 1316 mg; 3rd, 626 mg; and 4th, 608 mg) consisting mainly of piscerythrone and 6′-prenylpiscerythrone plus other minor isoflavonoids. The mother liquor was then taken to dryness, and the resulting solid (2.32 g) was adsorbed in EtOAc onto Florisil (30 g). After removing the EtOAc, the dry powder was chromatographed on a Florisil column (90 g) moistened with 5% H_2O (w/w) using mixtures of EtOAc in benzene as the eluting solvent. The fractions (each 100 ml) were eluted as follows: Fr–9–1 to Fr–9–3 (5% EtOAc in benzene), Fr–9–4 to Fr–9–6 (10% EtOAc), Fr–9–7 to Fr–9–9 (20% EtOAc), and Fr–9–10 to Fr–9–12 (40% EtOAc).

Small amounts of rotenone (**1**) were found in Fr–9–3 and –4, whilst larger quantities of ichthynone (**2**) and piscerythrone were distributed in Fr–9–6 to –8, and in Fr–9–10 to –12 respectively.

Fr–9–5 was concentrated and chromatographed (Si gel PTLC) in HEAm (30:20:1) to give bands fluorescing dull white (R_f 0.59) and dark yellow (R_f 0.36). Elution of the upper band yielded a pure amorphous powder (1.3 mg) identified as durmillone (**3**). Re-PTLC of the lower band in CM (80:1) gave ichthynone (**2**) at R_f 0.61 (1.7 mg) and 12a-hydroxyrotenone (**4**) at R_f 0.59 (25.8 mg). Further quantities of **4** were also found in Fr–9–5 and –6 (total 45.7 mg).

Fr–9–6 was concentrated to yield crystals of **2** (4.5 mg). The mother liquor was then subjected to PTLC in CM (70:1). A mixture of **2** and **4** was recovered from a broad band at R_f 0.3–0.6, and further amounts of **3** were found at R_f 0.67. A band at R_f 0.73 was eluted and purified by PTLC in HE (3:2) to give erythynone (**6**) as an oil (5.4 mg).

Fr–9–12 and Fr–9–13 were combined and chromatographed (PTLC) in CM (50:3). A band at R_f 0.69 was eluted and rechromatographed in CAAm (35:30:1) to yield an orange fluorescing compound (R_f 0.74). Elution and successive PTLC purification of this material in CM (50:1, R_f 0.67) and CAAm (40:10:1, organic layer; R_f 0.50) gave villosinol (**5;** 1.9 mg).

As reported earlier [3], fraction Fr–12 (eluted with 20% EtOAc in benzene) from the initial silica gel column, was concentrated and chilled to give precipitates (1st, 224 mg and 2nd, 618 mg chiefly consisting of **2** and piscidone). 12a-Hydroxyerythynone (**7**) was isolated as an oil (5.4 mg) from the mother liquor by PTLC in CM (50:2, R_f *ca.* 0.70), followed by elution and multiple development PTLC (× 4) in HE (3:2).

^{1}H NMR (500 MHz, J in Hz) data for derrone (**8**) *and alpinumisoflavone* (**9**)

Derrone (**8**) in $CDCl_3$: δ 1.478 (6H, s, 7″- and 8″-H_3), 5.591 (1H, d, J = 10.2, 5″-H), 6.296 (1H, s,

6-H), 6.685 (1 H, d, J = 10.2, 4″-H), 6.898 (2 H, d, J = 8.7, 3′- and 5′-H), 7.398 (2 H, d, J = 8.7, 2′- and 6′-H), 7.887 (1 H, s, 2-H), 12.904 (1 H, s, 5-OH). **8** in pyridine-d_5: δ 1.465 (6 H, s, 7″- and 8″-H_3), 5.636 (1 H, d, J = 10.0, 5″-H), 6.556 (1 H, s, 6-H), 6.713 (1 H, d, J = 10.0, 4″-H), 7.304 (2 H, d, J = 8.6, 3′- and 5′-H), 7.745 (2 H, d, J = 8.6, 2′- and 6′-H), 8.185 (1 H, s, 2-H), 11.886 (1 H, s, 4′-OH), 13.669 (1 H, s, 5-OH). *Alpinumisoflavone* (**9**) in $CDCl_3$: δ 1.473 (6 H, s, 7″- and 8″-H_3), 5.625 (1 H, d, J = 10.0, 5″-H), 6.336 (1 H, s, 8-H), 6.727 (1 H, d, J = 10.0, 4″-H), 6.893 (2 H, d, J = 8.3, 3′- and 5′-H), 7.394 (2 H, d, J = 8.3, 2′- and 6′-H), 7.819 (1 H, s, 2-H), 13.134 (1 H, s, 5-OH). **9** in pyridine-d_5: δ 1.455 (6 H, s, 7″- and 8″-H_3), 5.665 (1 H, d, J = 10.0, 5″-H), 6.503 (1 H, s, 8-H), 6.926 (1 H, d, J = 10.0, 4″-H), 7.294 (2 H, d, J = 8.4, 3′- and 5′-H), 7.718 (2 H, d, J = 8.4, 2′- and 6′-H), 8.127 (1 H, s, 2-H), 11.869 (1 H, s, 4′-OH), 13.915 (1 H, s, 5-OH).

Physicochemical properties of the isolate

Rotenone (**1**). $[\alpha]_D^{23}$ −148° (c = 0.25, MeOH). Osmate ester-pyridine complex in CH_2Cl_2, CD: $[\theta]_{476}^{23}$ − 4700 (*R*-configuration at E-ring) [12, 13]. CD $[\theta]_{max}^{23}$ rel. int. in MeOH: $[\theta]_{356}$ + 0.07, $[\theta]_{349}$ 0, $[\theta]_{334}$ − 0.07, $[\theta]_{327}$ − 0.04, $[\theta]_{307}$ − 0.58, $[\theta]_{300}$ − 0.44, $[\theta]_{280}$ − 1.00. MS (rel. int.): m/z 395 (M^+ + 1; 9), 394 (M^+; 35), 379 (3), 203 (4), 193 (15), 192 (100), 191 (26), 177 (15), 93 (3), 77 (3), 65 (3). ^{1}H NMR (acetone-d_6, 270 MHz, J = Hz): δ 1.76 (3 H, s, 8′-H_3), 2.95 (1 H, dd, J = 15.8 and 9.6, 4′-Ha), 3.30 (1 H, dd, J = 15.8 and 7.7, 4′-Hb), 3.64 (3 H, s, 3-OCH_3), 3.76 (3 H, s, 2-OCH_3), 3.90 (1 H, d, J = 3.8, 12a-H), 4.29 (1 H, d, J = 12.5, 6-Ha), 4.60 (1 H, dd, J = 12.5 and 3.3, 6-Hb), 4.93 (1 H, br. s, 7′-Ha), 5.08 (1 H, br. s, 7′-Hb), 5.14 (1 H, br. t-like, J = *ca.* 3.6, 6a-H), 5.35 (1 H, br. t-like, J = *ca.* 9, 5′-H), 6.46 (1 H, s, 4-H), 6.52 (1 H, d, J = 8.5, 10-H), 6.73 (1 H, s, 1-H), 7.78 (1 H, d, J = 8.5, 11-H).

Durmillone (**3**)

Amorphous powder. UV_{365} nm fluorescence: blue-white. Gibbs test: (−). MS (rel. int.): m/z 379 (M^+ + 1; 9), 378 (M^+; 35), 365 (4), 364 (26), 363 (M^+ − 15; 100), 348 (4), 217 (3), 182 (5), 181 (25), 175 (4), 146 (3), 145 (3). UV: λ_{max}, nm: MeOH 224 sh, 229, 264, 333, 347. The MeOH spectrum was unchanged with NaOMe, $AlCl_3$ or NaOAc. ^{1}H NMR (acetone-d_6, 270 MHz, J = Hz): δ 1.52 (6 H, s, 7″- and 8″-H_3), 3.93 (3 H, s, 6-OCH_3), 5.94 (1 H, d, J = 9.9, 5″-H), 6.04 (2 H, s, 1‴-H_2), 6.85 (1 H, d, J = 9.9, 4″-H), 6.91 (1 H, d, J = 8.0, 5′-H), 7.10 (1 H, dd, J = 8.0 and 1.7, 6′-H), 7.19 (1 H, d, J = 1.7, 2′-H), 7.48 (1 H, s, 5-H), 8.26 (1 H, s, 2-H). Comparative MS and ^{1}H NMR data for ichthynone (**2**) were as follows: MS (rel. int.): m/z 409 (M^+ + 1; 24), 408 (M^+; 100), 394 (19), 393 (M^+ − 15; 82), 379 (8), 378 (31), 377 (M^+ − 31; 95), 363 (10), 233 (13), 217 (6), 196 (44), 174 (33). ^{1}H NMR (acetone-d_6, 270 MHz, J = Hz): δ 1.52 (6 H, s, 7″- and 8″-H_3), 3.74 (3 H, s, 2′-OCH_3), 3.92 (3 H, s, 6-OCH_3), 5.93 (1 H, d, J = 9.9, 5″-H), 6.00 (2 H, s, 1‴-H_2), 6.77 (1 H, s, 3′-H), 6.85 (1 H, s, 6′-H), 6.85 (1 H, d, J = 9.9, 4″-H), 7.44 (1 H, s, 5-H), 8.10 (1 H, s, 2-H).

12a-Hydroxyrotenone (**4**)

Colourless semi-solid. UV_{365} nm fluorescence: yellow. $[\alpha]_D^{23}$ − 115° (c = 0.13, MeOH). Osmate ester-pyridine complex, CD: $[\theta]_{472}^{23}$ − 4300 (*R*-configuration at E-ring). CD $[\theta]_{max}^{23}$ rel. int. in MeOH: $[\theta]_{359}$ + 0.03, $[\theta]_{354}$ 0, $[\theta]_{334.5}$ − 1.00. MS (rel. int.): m/z 411 (M^+ + 1; 7), 410 (M^+; 24), 393 (3), 209 (15), 208 (100), 207 (40), 203 (13), 193 (9), 191 (3), 181 (7), 165 (11), 137 (3), 109 (4), 77 (4). UV: λ_{max}, nm: MeOH 237, 244 sh, 294, 315 sh (br.). The MeOH spectrum was unchanged with NaOMe, $AlCl_3$ or NaOAc. ^{1}H NMR (acetone-d_6, 270 MHz, J = Hz): δ 1.76 (3 H, s, 8′-H_3), 2.92 (1 H, dd, J = 15.7 and 9.6, 4′-Ha), 3.27 (1 H, dd, J = 15.7 and 8.0, 4′-Hb), 3.61 (3 H, s, 3-OCH_3), 3.77 (3 H, s, 2-OCH_3), 4.47 (1 H, dd, J = 12.2 and 1.1, 6-Ha), 4.59 (1 H, dd, J = 12.2 and 2.2, 6-Hb), 4.71 (1 H, br. dd, J = 2.2 and 1.1, 6a-H), 4.92 (1 H, br. s, 7′-Ha), 5.07 (1 H, br. s, 7′-Hb), 5.33 (1 H, t-like, J = *ca.* 9, 5′-H), 6.49 (1 H, s, 4-H), 6.53 (1 H, d, J = 8.8, 10-H), 6.64 (1 H, s, 1-H), 7.77 (1 H, d, J = 8.8, 11-H).

Villosinol (**5**)

Amorphous powder. UV_{365} nm fluorescence: white. Gibbs test: (−), dark brown. $[\alpha]_D^{23}$ − 38° (c = 0.053, MeOH) (lit. [16], −60.08°). Osmate ester-pyridine complex, CD: $[\theta]_{475}^{23}$ − 4900 (*R*-configuration at E-ring). CD $[\theta]_{max}^{23}$ rel. int. in MeOH: $[\theta]_{348}$ + 0.85, $[\theta]_{328}$ 0, $[\theta]_{303}$ − 1.00, $[\theta]_{290}$ − 0.63, $[\theta]_{281}$ − 0.76, $[\theta]_{255}$ 0. MS (rel. int.): m/z 427

($M^+ + 1$; 3), 426 (M^+; 12), 219 (14), 209 (13), 208 (100), 207 (37), 193 (7), 191 (3), 181 (5), 165 (9), 109 (3), 69 (5). UV: λ_{max}, nm: MeOH 234sh, 244sh, 299, 335sh; +NaOMe 248sh, 296, 370 (br.); +$AlCl_3$ 321; +NaOAc unchanged. ^{1}H NMR ($CDCl_3$, 270 MHz, J = Hz): δ 1.74 (3H, s, 8′-H_3), 2.83 (1H, dd, J = 15.4 and 7.7, 4′-Ha), 3.20 (1H, dd, J = 15.4 and 9.3, 4′-Hb), 3.76 (3H, s, 3-OC$\underline{H}_3$), 3.83 (3H, s, 2-OC$\underline{H}_3$), 4.46 (1H, d, J = 11.5, 6-Ha), 4.55 (1H, br. t-like, J = *ca.* 3, 6a-H), 4.58 (1H, dd, J = *ca.* 12 and 2.2, 6-Hb), 4.93 (1H, br. s, 7′-Ha), 5.05 (1H, br. s, 7′-Hb), 5.20 (1H, br. t, J = *ca.* 8.7, 5′-H), 6.04 (1H, s, 10-H), 6.49 (1H, s, 4-H), 6.70 (1H, s, 1-H), 11.82 (1H, s, 11-O$\underline{H}$). ^{1}H NMR (pyridine-d_5, 270 MHz, J = Hz): δ1.68 (3H, s, 8′-H_3), 2.85 (1H, dd, J = 15.4 and 7.7, 4′-Ha), 3.03 (1H, dd, J = 15.4 and 9.3, 4′-Hb), 3.48 (3H, s, 3-OC$\underline{H}_3$), 3.65 (3H, s, 2-OC$\underline{H}_3$), 4.9–5.0 (2H, m, 6-Ha and 6a-H), 4.87 (1H, dd, J = 12.4 and 2.5, 6-Hb), 5.03 (1H, br. s, 7′-Ha), 5.08 (1H, br. s, 7′-Hb), 5.16 (1H, br. t, J = *ca.* 8.5, 5′-H), 6.29 (1H, s, 10-H), 6.79 (1H, s, 4-H), 7.29 (1H, s, 1-H), 13.08 (1H, s, 11-O$\underline{H}$).

Erythynone (**6**)

Amorphous powder. UV_{365} nm fluorescence: brownish orange. Gibbs test: (−). $[\alpha]_D^{23}$ + 25° (c = 0.08, MeOH). CD $[\theta]_{max}^{23}$ rel. int. in MeOH: $[\theta]_{370}$ − 1.00, $[\delta]_{355sh}$ − 0.41, $[\theta]_{348}$ 0, $[\theta]_{340sh}$ + 0.62, $[\theta]_{335sh}$ + 0.65, $[\theta]_{332}$ + 0.68, $[\theta]_{331}$ 0, $[\theta]_{309}$ − 0.59, $[\theta]_{302}$ 0, $[\theta]_{290}$ + 1.00. MS (rel. int.): m/z 425 ($M^+ + 1$; 7), 424 (M^+; 30), 409 ($M^+ - 15$; 14), 393 (10), 379 (8), 233 (19), 217 (8), 193 (14), 192 (100), 191 (84), 179 (7), 177 (16), 147 (8). UV: λ_{max}, nm: MeOH 239sh, 248sh, 256, 264sh, 280sh, 325.5 (br.). The MeOH spectrum was unchanged with NaOMe, $AlCl_3$ or NaOAc. ^{1}H NMR (acetone-d_6, 270 MHz, J = Hz): δ 1.37 and 1.47 (both 3H, two s, 7′- and 8′-H_3), 3.65 (3H, s, 3-OC$\underline{H}_3$), 3.75 (3H, s, 2-OC$\underline{H}_3$), 3.80 (3H, s, 10-OC$\underline{H}_3$), 3.90 (1H, d, J = 3.8, 12a-H), 4.28 (1H, br. d, J = 12.2, 6-Ha), 4.64 (1H, dd, J = 12.3 and 3.3, 6-Hb), 5.08 (1H, br. t, J = *ca.* 4, 6a-H), 5.74 (1H, d, J = 9.9, 5′-H), 6.44 (1H, s, 4-H), 6.64 (1H, d, J = 9.9, 4′-H), 6.76 (1H, s, 1-H), 7.24 (1H, s, 11-H). NOE: Upon irradiation at δ 3.80 (10-OC$\underline{H}_3$), the signal at δ 7.24 (11-H) was enhanced.

12a-Hydroxyerythynone (**7**)

Colourless oil. UV_{365} nm fluorescence: brownish orange. Gibbs test: (−). $[\alpha]_D^{23}$ + 28° (c = 0.18, MeOH). CD $[\theta]_{max}^{23}$ rel. int. in MeOH: $[\theta]_{370.5}$ − 0.72, $[\theta]_{355}$ − 0.32, $[\theta]_{348}$ 0, $[\theta]_{332}$ + 0.48, $[\theta]_{312}$ 0, $[\theta]_{308}$ − 0.08, $[\theta]_{304}$ 0, $[\theta]_{286}$ + 1.00. MS (rel. int.): m/z 440 (M^+; 9), 423 (10), 422 ($M^+ - 18$; 39), 407 ($M^+ - 18 - 15$; 20), 379 (10), 233 (18), 217 (7), 209 (12), 208 (100), 207 (34), 192 (8), 149 (11), 81 (9), 71 (9), 69 (20). UV: λ_{max}, nm: MeOH 248sh, 256, 264sh, 276sh, 327 (br.), 346sh; +$AlCl_3$ 235, 246, 256, 324 (br.), 349sh. The MeOH spectrum was unchanged with $NaOCH_3$ or NaOAc. ^{1}H NMR (acetone-d_6, 270 MHz, J = Hz): δ 1.37 and 1.47 (both 3H, two s, 7′- and 8′-H_3), 3.62 (3H, s, 3-OC$\underline{H}_3$), 3.77 (3H, s, 2-OC$\underline{H}_3$), 3.80 (3H, s, 10-OC$\underline{H}_3$), 4.47 (1H, dd, J = 14.6 and 3.0, 6-Ha), 4.63 (1H, dd, J = 14.6 and 2.5, 6-Hb), 4.65 (1H, br. s, 6a-H), 5.74 (1H, d, J = 10.2, 5′-H), 6.47 (1H, s, 4-H), 6.60 (1H, d, J = 10.2, 4′-H), 6.68 (1H, s, 1-H), 7.22 (1H, s, 11-H).

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