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Isoaurostatin: total synthesis and structural revision☆

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Abstract—Isoaurostatin, a topoisomerase I inhibitor isolated from *Thermomonospora alba* has been synthesized for the first time from 2,4-dihydroxyacetophenone via 6-methoxybenzo-2(3*H*)-furanone in five steps. The *E*-isomer was converted into *Z*-isomer, but spectroscopic data of either of these two isomers did not match with those of the natural product. The structure of isoaurostatin has been revised to a known isoflavone, daidzein (**2**), based on careful analysis of spectroscopic data.

1. Introduction

Isoaurostatin, a novel topoisomerase I inhibitor was recently isolated from the culture filtrate of *Thermomonospora alba* strain No. 1520.[1] Isoaurostatin inhibited the relaxation activity of calf thymus topoisomerase I in a noncompetitive manner and did not inhibit the relaxation and decatenation of human placenta topoisomerase II. Structure of isoaurostatin was determined as **1** based on interpretation of spectroscopic data. Aurones and isoaurones, are naturally occurring yellow pigments of plants and are structurally related to flavonoids.[2] Aurones have limited natural occurrence and fewer methods of synthesis,[3–5] and naturally occurring isoaurones are extremely rare and only two compounds have been reported[6,7] so far. Structures of these compounds have been deduced on the basis of NMR data and confirmed by partial synthesis.[8,9] Pterocarposide, the only isoaurone C-glucoside reported so far, was isolated from *Pterocarpus marsupium* and its structure was deduced from spectroscopic data.[10] In this paper, we wish to report a total synthesis of the proposed structure of isoaurostatin (**1**) and its structural revision into daidzein (**2**).

2. Results and discussion

The general synthetic route to isoaurones involves the acid or base catalyzed condensation of substituted 2(3*H*)-benzofuranone with aromatic aldehydes. We, therefore, synthesized the desired 6-methoxybenzo-2(3*H*)-furanone (**9**) as follows. Willgerodt–Kindler reaction of 2-hydroxy-4-methoxyacetophenone (**7**) with sulfur and morpholine under phase transfer catalytic conditions[11] gave phenylacetic acid derivative **8**, in 66% yield. Lactonization of **8** in the presence of phosphorous oxychloride[12] yielded 6-methoxy-2(3*H*)-furanone (**9**) in 90% yield. Acid catalyzed condensation of **9** with 4-hydroxybenzaldehyde afforded 4′-hydroxy-6-methoxyisoaurone (**10**), which was demethylated using pyridine hydrochloride[13] to give 4′,6-dihydroxyisoaurone (**1**) in 63% yield (Scheme 1). The ^{1}H NMR spectrum showed that it is a mixture of two isomers and this was also confirmed by HPLC (*E*/*Z*—90:10). Attempts to purify further were not successful. In order to assign the stereochemistry unambiguously, we sought the *Z*-isomer also. Therefore, **1** was photoisomerised to **5** using a medium pressure mercury lamp. Again, it was obtained as a mixture of two isomers in the ratio of *Z*/*E*—90:10, after repeated crystallizations.

In (*E*) and (*Z*)-isoaurones, the configuration was assigned based on the differences in chemical shifts of olefinic protons (H-10) and *ortho* protons (H-2′ and H-6′) of the pendant aryl unit. These protons are deshielded by the carbonyl group and are expected to give downfield signals.[14] In *E*-isomers, H-2′, H-6′ protons of the aryl unit appear as doublet in the range of δ 7.0–7.8, whereas in *Z*-isomers the corresponding protons appear in the range of δ 8.0–8.2.[14] The chemical shifts of synthetic **1**, gave a doublet at δ 7.65 (H-2′, H-6′) supporting the *E*-configuration and in **5**, these protons resonated at δ 8.16 (H-2′, H-6′) confirming a *Z*-configuration. The olefinic protons (H-10) in

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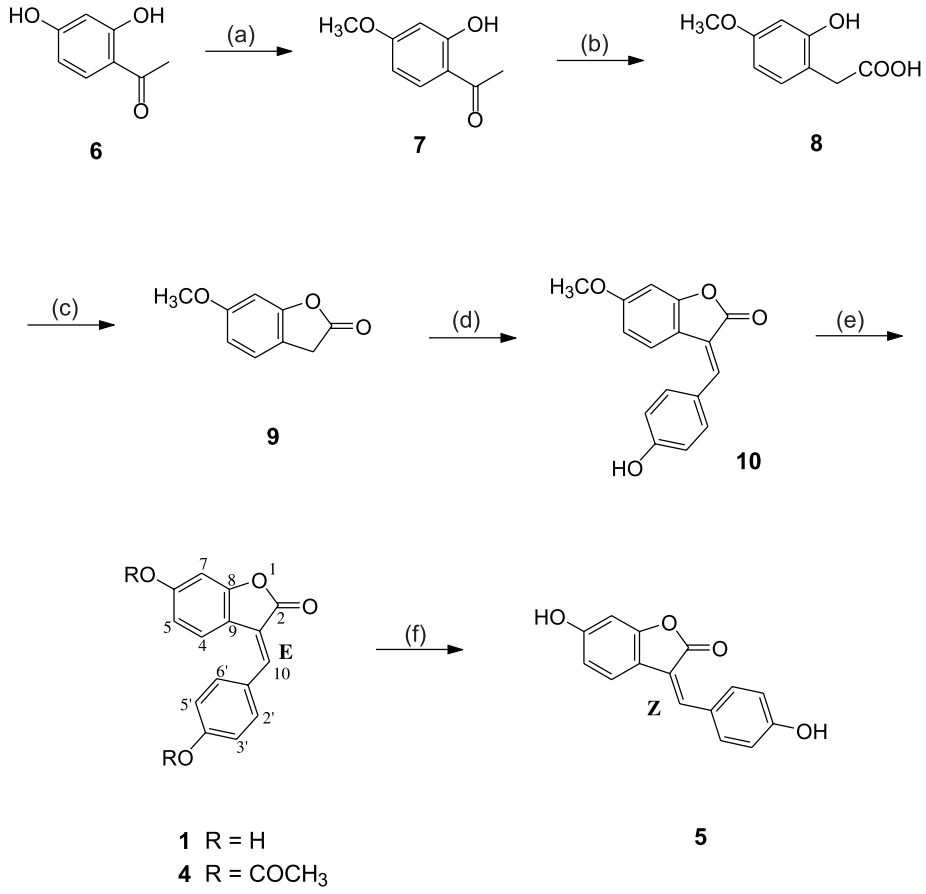

Scheme 1. Reagents and conditions: (a) DMS, K_2CO_3, acetone, rt, 5 h, 91%; (b) S, morpholine, *p*-TSA, 20% NaOH, PTC, reflux, 16 h, 66%; (c) $POCl_3$, DCE, rt, 15 h, 90%; (d) 4-hydroxybenzaldehyde, Ac_2O, 90 °C, 3 h, 61%; (e) pyridine HCl, 180–190 °C, 3 h, 63%; (f) UV, THF, rt, 10 h, 70%.

Table 1. ^1H and ^{13}C NMR data of **1**, **5** and isoaurostatin[1]

Position	**1**-*E*[a]		**5**-*Z*[a]		Isoaurostatin[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		169.4		166.5		174.8
3		117.8		116.9		123.6
4	7.69 (d, 8.5)	123.4	7.58 (d, 8.3)	125.2	7.94 (d, 8.5)	127.3
5	6.58 (d, 8.5)	111.1	6.63 (dd, 2.1, 8.3)	111.1	6.91 (dd, 2.4, 8.5)	115.3
6		159.9		160.3		162.8
7	6.64 (s)	98.5	6.59 (d, 2.1)	97.9	6.83 (d, 2.4)	102.2
8		154.9		152.9		157.6
9		112.9		116.3		116.6
10	7.50 (s)	137.1	7.72 (s)	137.9	8.25 (s)	152.9
1′		124.8		120.6		122.6
2′,6′	7.65 (d, 8.4)	132	8.16 (d, 8.7)	134.3	7.36 (d, 8.5)	130.1
3′,5′	6.92 (d, 8.4)	115.8	6.88 (d, 8.7)	115.5	6.79 (d, 8.5)	115
4′		159.8		159		157.2

[a] ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) in DMSO-d_6.
[b] ^1H NMR data (500 MHz) and ^{13}C NMR data (125 MHz) in DMSO-d_6 are taken from Ref. 1.

these isomers gave a singlet at δ 7.50 (for *E*-isomer) and at δ 7.72 (for *Z*-isomer).

However, the olefinic proton in isoaurostatin was reported to resonate at δ 8.25 and the other proton and carbon NMR chemical shifts (Table 1) are also not consistent with either *E*-isomer **1** or *Z*-isomer **5**. Furthermore, the proton NMR data of acetyl derivative **4** of the *E*-isomer are also not consistent with the reported data on isoaurostatin diacetate.[1] Obviously, isoaurostatin does not posses an isoaurone structure. So, we have carefully reanalyzed the spectroscopic

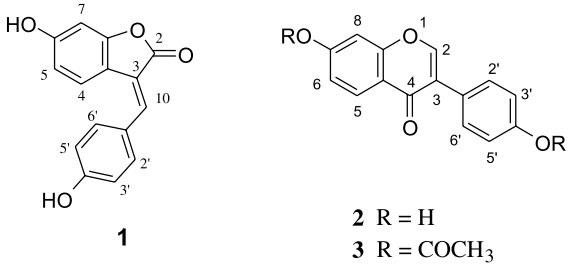

Figure 1. Structures of isoaurostatin (**1**) and daidzein (**2**).

Table 2. ^{1}H and ^{13}C NMR data of reassigned isoaurostatin, isoaurostatin diacetate, daidzein (**2**) and daidzein diacetate (**3**)

Position	Isoaurostatin[a]		Isoaurostatin diacetate[b]	Daidzein (**2**)[c]		Daidzein diacetate **3**[d]
	δ_H	δ_C	δ_H	δ_H	δ_C	δ_H
OAc			2.32 (s), 2.36 (s)			2.32 (s), 2.37 (s)
2	8.25 (s)	152.9	8.01 (s)	8.28 (s)	152.3	8.01 (s)
3		123.6			122.7	
4		174.8			178.6	
5	7.94 (d, 8.5)	127.3	8.32 (d, 8.5)	7.95 (d, 8.7)	127.2	8.33 (d, 8.7)
6	6.91 (dd, 2.4, 8.5)	115.3	7.18 (dd, 1.8, 8.5)	6.92 (dd, 2.1, 8.7)	115.1	7.18 (dd)[e]
7		162.8			162.6	
8	6.83 (d, 2.4)	102.2	7.32 (d, 1.8)	6.84 (d, 2.1)	102.2	7.32 (d, 2.1)
9		157.6			157.6	
10		116.6			116.9	
1′		122.6			123.9	
2′,6′	7.36 (d, 8.5)	130.1	Not reported	7.36 (d, 8.6)	130	7.59 (d, 8.6)
3′,5′	6.79 (d, 8.5)	115	7.17 (d, 8.5)	6.79 (d, 8.6)	115.1	7.17 (d, 8.6)
4′		157.2			157.3	

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in DMSO-d_6 are taken from Ref. 1 and reassigned.
[b] ^{1}H NMR (500 MHz) data in $CDCl_3$ is taken from Ref. 1 and reassigned.
[c] ^{1}H NMR (300 MHz) in DMSO-d_6 and ^{13}C NMR (15 MHz) in $CDCl_3$/DMSO-d_6 are taken from Refs. 15 and 16.
[d] ^{1}H NMR (400 MHz) in $CDCl_3$.
[e] Merged with H-3′,5′ doublet.

data reported for natural isoaurostatin and found that the data agrees well with an isomeric structure **2** (Fig. 1). The carbonyl absorptions in IR for isoaurostatin was reported at 1630 cm^{-1}, which is in good agreement with those of reported for **2**, but not to the isoaurones ($\sim$1750 cm^{-1}). Further, the singlet at δ 8.25 in the ^{1}H NMR data of isoaurostatin agrees well with the chemical shift reported for H-2 in **2**, but not to the olefinic proton in isoaurones (δ 7.0–7.8).[10,14] Based on the above, the proton and carbon NMR data reported for isoaurostatin have been reassigned and the data are found to be in good agreement with the reported proton[15] and carbon[16] NMR data of daidzein (**2**) (Table 2). Furthermore, the reassigned proton NMR data reported for isoaurostatin diacetate are also identical with those of daidzein diacetate, **3** (Table 2) prepared by us. From the foregoing, the novel inhibitor of topoisomerase I isolated from the culture filtrate of *Thermomonospora alba* is, in fact, daidzein (**2**), a known isoflavone.

3. Conclusions

In summary, we have accomplished the first total synthesis of the proposed structure of isoaurostatin (**1**), a novel topoisomerase I inhibitor from *Thermomonospora alba* in five steps starting from 2,4-dihydroxyacetophenone. The *E*-isomer **1** was converted into *Z*-isomer **5** and the spectral data of these isomers did not match with those reported for isoaurostatin. The reported spectral data of natural product have been reassigned and found to match well with those recorded for daidzein (**2**).

4. Experimental

4.1. General

Melting points were recorded on a Mel-Temp melting point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin–Elmer BX1 FTIR Spectrophotometer. ^{1}H NMR (400 MHz), ^{13}C NMR-DEPT (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer using TMS as internal reference and the values for chemical shifts (δ) being given in ppm and coupling constants (J) in Hertz (Hz). Mass spectra were recorded on Agilent 1100 LC/MSD. HPLC was recorded by a Shimadzu SCL-10A instrument under the following conditions: column, Altima C18; flow rate, 1 mL/min; detection at 384 nm; mobile phase, 0.1% phosphoric acid: acetonitrile (65:35, v/v); retention time for **1**, 20.91 and for **5**, 22.51 min. Acme silica gel G and silica gel (100–200 mesh) were used for analytical TLC and column chromatography, respectively.

4.1.1. 2-Hydroxy-4-methoxyacetophenone (7). A mixture of **6** (6 g, 39.5 mmol), dimethyl sulfate (4.1 mL, 43.4 mmol), potassium carbonate (8.2 g, 59.2 mmol) and acetone (100 mL) was stirred at rt for 5 h. After completion of reaction, the solid was filtered off and the solvent was evaporated. The residue obtained was chromatographed over silica gel column using mixtures of petroleum ether–ethyl acetate (90:10) as eluent to give **7** (6 g, 91%) as a white solid, mp 46–48 °C (lit.[17] mp 49–50 °C) and its spectroscopic data are consistent with those reported in the literature.[17]

4.1.2. 2-(2-Hydroxy-4-methoxyphenyl)acetic acid (8). A mixture of **7** (1.66 g, 10 mmol), sulfur (0.64 g, 20 mmol), morpholine (3 mL, 30 mmol) and *p*-toluenesulfonic acid (0.06 g, 0.32 mmol) was refluxed under constant stirring at 120–130 °C for 8 h. After completion of reaction, the mixture was allowed to cool and 20% NaOH (10 mL) and tetrabutylammonium bromide (16 mg, 0.05 mmol) were added and continued hydrolysis for further 8 h at 100 °C. The cooled reaction mixture was filtered and the filtrate was acidified with HCl to pH 2. The precipitated solid was filtered and chromatographed over silica gel column using hexane–EtOAc (80:20) as eluents to give **8** (1.2 g, 66%) as a light yellow solid, mp 130–132 °C (lit.[18] mp 130 °C) and its spectroscopic data are consistent with those reported in the literature.[18]

4.1.3. 6-Methoxy-3-hydrobenzo[*b*]furan-2-one (9). A mixture of **8** (340 mg) and phosphorous oxychloride (1.5 mL) in dichloroethane (10 mL) was stirred at rt for 15 h. The reaction mixture was diluted with water (50 mL) and extracted with chloroform (3×30 mL) and the combined chloroform layer was washed with water (2× 20 mL), sodium bicarbonate (20 mL) and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using petroleum ether–EtOAc (90:10) as eluents to give **9** (270 mg, 90%) as a light yellow solid, mp 58–60 °C (lit.[18] mp 55–56 °C) and its spectroscopic data are consistent with those reported in the literature.[18]

4.1.4. 3-[(4-Hydroxyphenyl)methylene]6-methoxybenzo-[*b*]furan-2-one (10). A mixture of **9** (0.5 g, 3.05 mmol), 4-hydroxybenzaldehyde (0.372 g, 3.05 mmol) in acetic anhydride (7.5 mL, 79.4 mmol) and triethylamine (0.5 mL) was heated at 90 °C for 2 h. The cooled reaction mixture was poured into ice-cooled water (50 mL) and extracted with diethyl ether (3×30 mL). The organic layer was washed with water (2×20 mL), brine (20 mL) and dried over sodium sulfate, and the solvent was removed under vaccum. The residue was dissolved in methanol (10 mL), HCl (20%, 10 mL) and refluxed for 2 h. The cooled reaction mixture was poured into ice-cooled water (50 mL) and extracted with ethyl acetate (3×30 mL). The organic layer was washed with water (2×20 mL), brine (20 mL) and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using petroleum ether–EtOAc (80:20) to give **10** (500 mg, 61%), which was recrystallized from chloroform to give the product as a yellow crystalls, mp 152–154 °C; ν_{max} (KBr): 3310, 1743, 1594, 1286, 1226, 1074, 967, 826 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.83 (3H, s, $-OCH_3$), 6.76 (1H, d, J=8.5 Hz, H-5), 6.95 (2H, d, J=8.2 Hz, H-3′,5′), 6.96 (1H, s, H-7), 7.60 (1H, s, =CH), 7.70 (2H, d, J=8.2 Hz, H-2′,6′), 7.79 (1H, d, J=8.5 Hz, H-5), 10.30 (1H, brs, Ar-OH); MS (ESI, negative scan): *m/z* 267 $(M-H)^-$. HRMS (*m/z*): Calcd for $C_{16}H_{12}O_4$ (M+Na): 291.0633. Found: 291.0636.

4.1.5. 6-Hydroxy-3-[(4-hydroxyphenyl)methylene]benzo-[*b*]furan-2-one (1*E*). A mixture of **10** (100 mg) and pyridine hydrochloride (1.5 g) was stirred at 180–190 °C for 3 h. The cooled reaction mixture was diluted with water (20 mL), acidified with dil HCl and extracted with EtOAc (3×20 mL). The combined EtOAc layer was washed with brine (20 mL) and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using chloroform–methanol (94:6) as eluents to give **1**-*E* (60 mg, 63%), which was recrystallized from chloroform–methanol to give the product as a yellow crystalls, *E*/*Z*–90:10. Mp 258–260 °C; ν_{max} (KBr): 3350, 1733, 1582, 1374, 1280, 1241, 1069, 959 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6): see Table 1; MS (ESI, negative scan): *m/z* 253 $(M-H)^-$. HRMS (*m/z*): Calcd for $C_{15}H_{10}O_4$ (M+Na): 277.0477. Found: 277.0484.

4.1.6. 6-Acetyloxy-3-[(4-acetyloxyphenyl)methylene]-benzo[*b*]furan-2-one (4). A mixture of **1** (20 mg), acetic anhydride (1 mL), and pyridine (1 mL) was kept standing at room temperature for 16 h and diluted with diethyl ether (20 mL). The mixture was washed successively with water (20 mL), dil HCl (20 mL), water (20 mL) and brine (20 mL) and dried over sodium sulfate. The solution was filtered and the residue obtained after evaporation of the solvent was chromatographed over silica gel column using hexane–EtOAc (80:20) as eluents to give **4** (20 mg) as a light yellow solid, mp 128–130 °C; ν_{max} (neat): 1767, 1617, 1598, 1195, 1168, 1118, 1078, 1013 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.32 (3H, s, OAc), 2.35 (3H, s, OAc), 6.80 (1H, dd, J=2.2, 8.4 Hz, H-5), 6.96 (1H, d, J=2.2 Hz, H-7), 7.24 (2H, d, J= 8.7 Hz, H-3′,5′), 7.70 (2H, d, J=8.7 Hz, H-2′,6′), 7.73 (1H, d, J=8.4 Hz, H-4), 7.81 (1H, s, H-10); ^{13}C NMR ($CDCl_3$): δ 169.1, 168.9, 168.6, 155.0, 152.4, 152.2, 139.7, 131.5, 130.7, 123.3, 122.3, 121.6, 119.4, 117.1, 105.8, 21.2, 21.1; MS (ESI, positive scan): *m/z* 339 $(M+H)^+$. Analysis found: C, 67.38; H, 4.21%. Calcd for $C_{19}H_{14}O_6$: C, 67.45; H, 4.17%.

4.1.7. 6-Hydroxy-3-[(4-hydroxyphenyl)methylene]benzo-[*b*]furan-2-one (5*Z*). A solution of **1** (100 mg) in THF (90 mL) was irradiated using a medium pressure mercury lamp for 10 h and the residue obtained after evaporation of the solvent was recrystallized from chloroform–methanol to give **5** (70 mg, 70%) as a yellow solid, *Z*/*E*—90:10; mp 258–260 °C; ^1H and ^{13}C NMR (DMSO-d_6): see Table 1; MS (ESI, negative scan): *m/z* 253 $(M-H)^-$.

4.1.8. Daidzein diacetate (3). A mixture of daidzein (96%, 40 mg), acetic anhydride (1 mL), and pyridine (1 mL) was kept standing at room temperature for 16 h and diluted with diethyl ether (20 mL). The mixture after usual work-up as described above, gave diacetate **3** (45 mg, 85%) as a white solid, mp 186–188 °C (lit.[19] mp 188–190 °C); ν_{max} (neat): 1750, 1644, 1616, 1222, 1017 cm^{-1}; ^1H NMR ($CDCl_3$): see Table 2; MS (ESI, positive scan): *m/z* 339 $(M+H)^+$.

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