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TWO 2-ARYLBENZOFURANS AS INSECT FEEDING DETERRENTS FROM SAINFOIN (*ONOBRYCHIS VICIIFOLIA*)

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Key Word Index—*Onobrychis vicifolia*, Leguminosae, sainfoin, 2-arylbenzofuran, sainfuran, methylsainfuran, antifungal activity, insect feeding deterrent, *Costelytra zealandica*

Abstract—Two 2-arylbenzofurans have been isolated as insect feeding deterrents from the roots of the forage legume, sainfoin They have been identified as 2-(2′-hydroxy-4′-methoxyphenyl)-5-hydroxy-6-methoxybenzofuran (sainfuran) and 2-(2′,4′-dimethoxyphenyl)-5-hydroxy-6-methoxybenzofuran (methylsainfuran) Their synthesis is described

INTRODUCTION

In recent studies on the susceptibility of pasture plants to the subterranean larvae of the beetle, *Costelytra zealandica* White (Coleoptera Scarabaeidae), several legumes including the forage legume, sainfoin (*Onobrychis vicifolia* Scop), were resistant to the insect [1, Farrell, personal communication] Extracts of the roots of resistant legumes have been found to deter feeding by 3rd instar *C zealandica* larvae when tested in a standard feeding assay [2, 3] and similar feeding deterrent activity was found from root extracts of sainfoin In common with other resistant legumes [4, 5], it was the phenolic compounds from the root extracts which accounted for most of the feeding deterrent activity of the plant and we wish to report here the structure of two 2-arylbenzofurans, isolated as insect feeding deterrents from sainfoin They have been given the common names sainfuran (**1**) and methylsainfuran (**2**)

In a previous examination [6] of the antifungal compounds elicited in response to fungal infection, the leaves of sainfoin were found to produce a number of isoflavonoids including vestitol and medicarpin, already recognized as phytoalexins and insect feeding deterrents [5] Although 2-arylbenzofurans have been isolated as phytoalexins from several plants [7–11], they were not recognized in the leaf exudates of sainfoin

RESULTS AND DISCUSSION

Extraction and solvent partition of sainfoin roots gave an ether phase which significantly ($p < 0\,05$, Wilcoxon's Rank Sum Test) reduced larval feeding of *C zealandica* in the standard feeding assay [2, 3] Repeated chromatography of the residue gave a series of fractions, each of which showed significant feeding deterrent activity and two compounds were finally obtained crystalline On TLC, both compounds exhibited a blue fluorescence under UV light and when the plates were sprayed with tetrazotised *o*-dianisidine (Fast Blue B), purple–brown spots were obtained The UV spectra of the two compounds were characteristic of 2-arylbenzofurans [7, 8] with maxima at 326 and 342 nm which shift in alkali to 346 and 355 nm respectively

The mass spectra of the two benzofurans were simple, with molecular ions at m/z 286 (**1**) and 300 (**2**) respectively, corresponding to molecular formulae of $C_{16}H_{14}O_5$ and $C_{17}H_{16}O_5$ These compositions indicated that sainfuran (**1**) had two hydroxyl and two methoxyl groups attached to the 2-arylbenzofuran skeleton, while methylsainfuran (**2**) had one hydroxyl and three methoxyl groups This was confirmed by the preparation of a diacetate (**3**) from **1** and a monoacetate (**4**) from **2** and from the ^{1}H NMR spectra which showed two methoxyl signals at δ 3 79 and 3 92 for **1** and three methoxyl signals at δ 3 86, 3 92 and 4 01 for **2**

A detailed analysis of the ^{1}H NMR spectra, including spin simulation of the aromatic region, identified the benzofurans as **1** and **2** Both spectra showed three sets of peaks in the aromatic region—a singlet, an AB subspectrum and an ABC subspectrum The low field double doublets at δ 7 80 (**1**) and δ 7 88 (**2**) formed the typical 'C' portion of the ABC multiplet and the chemical shifts are consistent with reported values for a C-6′ proton of 2-arylbenzofurans [12]. Spectral simulation indicated that the A and B protons of this multiplet were *ortho* and *para* to H-6′ (J = 8, 1 Hz) and *meta* to each other (J = 2 5 Hz) Therefore, the *O*-substitution pattern of ring A, in both compounds, can only be 2′, 4′ In benzofurans a long range coupling (0 8–0 9 Hz) exists between the C-7 and C-3 protons [12] This coupling was apparent in the AB subspectrum of methylsainfuran (**2**) and the peaks at δ 7 09 and 7 15 can be assigned to H-7 and H-3 respectively In sainfuran (**1**) the chemical shifts of the two protons were almost coincident and the signals appeared as singlets (δ 7 15 and 7 17) with the outer lines of the AB subspectrum not observed The remaining singlet in the spectrum of both compounds (δ 7 00 and δ 6 99) was assigned to H-4 which has been observed as a singlet in benzofurans of similar substitution [9, 13]

Having established the substitution pattern, the position of the methoxyl groups can be deduced from a comparison of ^{1}H NMR spectra of the acetates In the spectrum of sainfuran (**1**) all the aromatic protons are displaced on acetylation indicating the presence of an OH

group on both rings A and B On the other hand, only ring B protons are shifted in the spectrum of methylsainfuran monoacetate showing 4 (and 2) has methoxyls at C-2′ and C-4′ The acetylation shifts of H-3′, H-5′ and H-6′ in 1 allow the hydroxyl group to be assigned *para* to H-5′, since this shows the greater displacement (Δ0 39 ppm) and *ortho* to H-3′ (displacement Δ0 25 ppm) [14] This assignment for sainfuran (1) is supported by a positive test to the Gibbs reagent The C-4 proton of 1 and 2 is displaced by Δ0 29 ppm and Δ0 26 ppm, respectively, on acetylation indicating that the hydroxyl group in ring B must be *ortho* at C-5 The remaining methoxyl group for both compounds must be on C-6

The structures of sainfuran and methylsainfuran were confirmed by synthesis This entailed chemical modification of a suitable isoflavone (8), a route which was used successfully to make isopterofuran [11] 2′,5′-Dibenzyloxy-4′,7-dimethoxyisoflavone (9) was synthesized *via* $Tl(NO_3)_3$ oxidation of the corresponding chalcone 7, prepared by standard methods Base hydrolysis of the isoflavone gave the deoxybenzoin, 10, which was methylated to give 11 Catalytic hydrogenation of 10 and 11 in acid solution gave 1 and 2 which were identical to the natural products

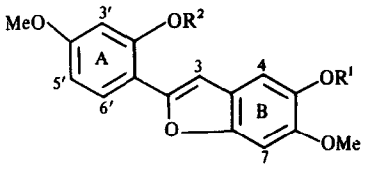
The two benzofurans showed antifungal and insect feeding deterrent activity In a TLC bioassay [15] they inhibited spore germination of *Cladosporium cladosporoides* and when incorporated into agar–cellulose medium at 2 μg/g, the feeding of *Costelytra zealandica* larvae was significantly reduced

EXPERIMENTAL

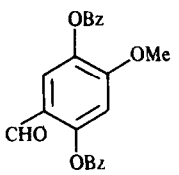
Mps were measured using a Kofler block and are uncorr TLC was carried out on Merck precoated plates (silica gel 60 F_{254}) with petrol–Et_2O (1 5) and the compounds were visualized by spraying with tetrazotized *o*-dianisidine soln (0 25%, Fast Blue B) ^1H NMR were measured at 80 MHz (Varian FT 80-A) with TMS as internal standard MS were obtained on AEI MS 30 or AEI MS 902 using a direct insertion probe at 70 eV

Insect feeding deterrent assays These were performed as described previously [2] Field-collected 3rd instar larvae of *Costelytra zealandica* which had been starved for 24 hr were enclosed individually in Petri dishes with a 4% agar–4% cellulose powder disc (1 5 cm) containing a standard feeding stimulant (0 1 M sucrose + 0 01 M ascorbic acid) plus the test plant material Feeding deterrent activity was assessed by comparing 24 hr faecal pellet counts of larvae offered discs containing the test material (TC) with similar counts of larvae offered standard discs containing feeding stimulants only (C) and of a third group offered blank discs prepared with water Twenty larvae were tested with each medium Wilcoxon's Rank Sum test was used to determine whether TC was significantly less than C ($p < 0.05$)

Isolation of constituents The dried roots (3 kg) of sainfoin



1 $R^1 = R^2 = H$
2 $R^1 = H, R^2 = Me$
3 $R^1 = R^2 = Ac$
4 $R^1 = Ac, R^2 = Me$



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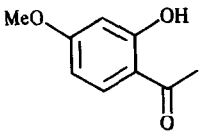

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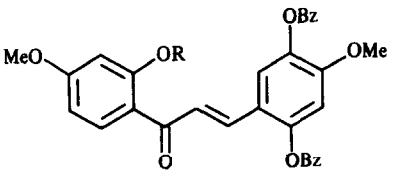
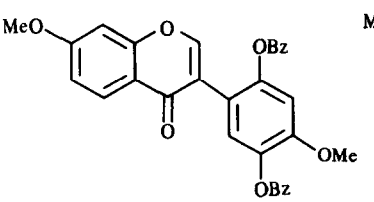


R
7 H
8 Ac



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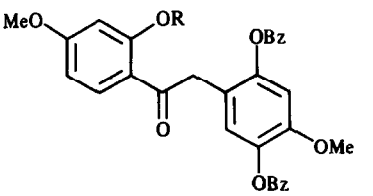

R
10 H
11 Me

(*Onobrychis viciifolia*) were milled and extracted by continuous percolation with EtOH The EtOH extract was concd *in vacuo* to a small vol and partitioned between petrol and MeOH–H_2O (4 1) The aq phase was concd *in vacuo* and repartitioned between Et_2O–H_2O The Et_2O phase was concd *in vacuo* and the residue (50 g) was percolated through Al_2O_3 (2 kg 10% H_2O) with EtOAc and 10 × 1 l fractions were collected Fractions 1–5 were combined and re-eluted through silica gel (500 g) with CH_2Cl_2 collecting 30 × 200 ml fractions Those fractions (4–6, 7–17) showing spots of the same R_f and staining purple–brown with Fast Blue B were combined Fractions 4–6 were chromatographed in silica gel with petrol–Et_2O (9 1) to give **2** (50 mg, R_f 0 66) Fractions 7–17 were chromatographed on Sephadex LH_{20} with CH_2Cl_2 and those fractions containing benzofuran were rechromatographed on silica gel with CH_2Cl_2–EtOH (9 1) to give pure **1** (70 mg, R_f 0 29)

2-(2′-*Hydroxy*-4′-*methoxyphenyl*)-5-*hydroxy*-6-*methoxybenzofuran* (*sainfuran*, **1**) Mp 150–152° (MeOH) Gibbs test blue–green, λ_{max} nm 656 Found M 286 0833, $C_{16}H_{14}O_5$ requires 286 0841 λ_{max}^{EtOH} nm 213 (83%), 243 sh (24%), 251 sh (20%), 275 (41%), 284 (45%), 291 (32%), 311 sh (59%), 326 (100%), 342 (99%) $\lambda_{max}^{EtOH+NaOH}$ nm 213 (100%), 248 sh (56%), 257 (55%), 277 sh (48%), 284 (50%), 315 sh (37%), 328 sh (52%), 346 (80%), 355 sh (75%) MS *m/z* (rel int) 286 (M, 59), 271 (100), 143 (18) ^{1}H NMR [$(CD_3)_2CO$, 80 MHz] δ 3 79 (*s*, OMe), 3 92 (*s*, OMe), 6 59 (*dd*, *J* = 8, 2 5 Hz, H-5′), 6 61 (*dd*, *J* = 2 5, 1 Hz, H-3′), 7 00 (*s*, H-4), 7 15 (*s*, H-7 or H-3), 7 17 (*s*, H-3 or H-7), 7 80 (*dd*, *J* = 8, 1 Hz, H-6′) *Diacetate* (**3**, pyridine–Ac_2O, 2 1) mp 160–161° MS *m/z* (rel int) 370 (M, 27), 328 (39), 287 (19), 286 (100), 285 (10), 272 (17), 271 (92) ^{1}H NMR [$(CD_3)_2CO$, 80 MHz] δ 2 25 (*s*, OAc), 2 40 (*s*, OAc), 3 86 (*s*, OMe), 3 88 (*s*, OMe), 6 86 (*dd*, *J* = 2 5, 0 2 Hz, H-3′), 6 98 (*dd*, *J* = 8 8, 2 5 Hz, H-5′), 7 29 (*s*, H-4), 7 31 (*s*, H-7 or H-3), 7 32 (*s*, H-3 or H-7), 7 88 (*dd*, *J* = 8 8, 0 2 Hz, H-6′)

2-(2′,4′-*Dimethoxyphenyl*)-5-*hydroxy*-6-*methoxybenzofuran* (*methylsainfuran*, **2**) Mp 147–148° (MeOH) Gibbs test, no reaction Found M 300 1005, $C_{17}H_{16}O_5$ requires 300 0998 λ_{max}^{EtOH} (nm) 211 (80%), 242 sh (32%), 251 sh (17%), 275 (37%), 284 (42%), 298 (34%), 312 sh (59%), 326 (100%), 342 (98%) $\lambda_{max}^{EtOH+NaOH}$ nm 211 (100%), 245 sh (18%), 282 sh (42%), 291 (46%), 317 (38%), 346 (55%), 355 sh (52%) MS *m/z* (rel int) 300 (M, 77), 285 (100), 257 (3), 242 (8), 227 (8), 150 (20) ^{1}H NMR [$(CD_3)_2CO$, 80 MHz] δ 3 86 (*s*, OMe), 3 92 (*s*, OMe), 4 01 (*s*, OMe), 6 66 (*dd*, *J* = 8, 2 5 Hz, H-5′), 6 70 (*dd*, *J* = 2 5, 1 Hz, H-3′), 6 99 (*s*, H-4), 7 09 (*d*, *J* = 0 8 Hz, H-7 or H-3), 7 15 (*d*, *J* = 0 8 Hz, H-3 or H-7), 7 85 (*dd*, *J* = 8, 1 Hz, H-6′) *Acetate* (**4**, pyridine–Ac_2O, 2 1) mp 138–139° Found M 342 1103, $C_{19}H_{18}O_6$ requires 342 1103 MS *m/z* (rel int) 342 (M, 52), 301 (13), 300 (74), 286 (17), 285 (100), 242 (4), 227 (4) ^{1}H NMR [$(CD_3)_2CO$, 80 MHz] δ 2 25 (*s*, OAc), 3 87 (*s*, OMe), 3 88 (*s*, OMe), 4 01 (*s*, OMe), 6 68 (*dd*, *J* = 8, 2 3 Hz, H-5′), 6 71 (*dd*, *J* = 2 3, 1 Hz, H-3′), 7 16 (*d*, *J* = 0 8 Hz, H-7 or H-3), 7 25 (*s*, H-4), 7 28 (*d*, *J* = 0 8 Hz, H-3 or H-7), 7 88 (*dd*, *J* = 8, 1 Hz, H-6′)

2,5-*Dibenzyloxy*-4-*methoxybenzaldehyde* (**5**) Vanillin was oxidized to methoxyquinol by a modification of the procedure described by Surrey [16] Mp 85–86°, lit mp 89° [17] Methoxyquinol was converted by the Gatterman synthesis to 2,5-dihydroxy-4-methoxybenzaldehyde [18], mp 203–205° (lit mp 204–206° [19]), which was benzylated to give **5**, mp 129–131° (lit mp 123–124° [19]) MS *m/z* (rel int) 348 (M, 5), 258 (2), 257 (2), 168 (4), 167 (5), 91 (100) ^{1}H NMR ($CDCl_3$) δ 3 83 (*s*, OMe), 5 03 (2H, *s*, $ArCH_2$), 5 10 (2H, *s*, $ArCH_2$), 6 50 (1H, *s*, ArH), 7 34 (11H, *br s*, ArH), 9 85 (1H, *s*, CHO)

2,5-*Dibenzyloxy*-4,4′-*dimethoxy*-2′-*acetyoxychalcone* (**8**) A suspension of 2-hydroxyl-4-methoxyacetophenone (**6**, 2 6 g) and **5** (5 g) in *n*-BuOH (75 ml) was refluxed with 25% NaOH (w/w, 150 ml) for 10 hr and stirred for a further 12 hr Steam distillation of the reaction mixture gave a dark yellow oil-solid which was recrystallized from HOAc to give the chalcone **7**, 3 g, mp 202–206° Found M 496 1878, $C_{37}H_{28}O_6$ requires 496 1885 MS *m/z* (rel int) 496 (M, 1), 91 (100) ^{1}H NMR ($CDCl_3$) δ 3 83 (*s*, OMe), 3 90 (*s*, OMe), 5 12 (4H, *s*, $ArCH_2$), 6 4–6 6 (2H, *m*, CH=CH), 7 09–7 86 (15H, *m*, ArH) The chalcone **7** (3 g) was suspended in dry pyridine–Ac_2O (45 ml, 2 1) for 18 hr to give **8**, mp 147–149° MS *m/z* (rel int) 538 (M, 3), 405 (5), 191 (19), 151 (15), 91 (100) ^{1}H NMR ($CDCl_3$): δ 2 21 (*s*, OAc), 3 84 (*s*, OMe), 3 85 (*s*, OMe), 5 09 (4H, *s*, $ArCH_2$), 6 54–6 78 (2H, *m*, CH=CH), 7 03–7 96 (15H, *m*, ArH)

2′,5′-*Dibenzyloxy*-4′,7-*dimethoxyisoflavone* (**9**) A suspension of **8** (2 g) in MeOH (800 ml) was stirred with Tl $(NO_3)_3$ $3H_2O$ (3 g) for 18 hr when KOH (4 g) was added and the mixture stirred for 1 hr After neutralisation with conc HCl, 10% HCl (100 ml) was added and the mixture refluxed for 1 hr Extraction with EtOAc and concn *in vacuo* gave **9** (1 77 g), mp 132–134° (MeOH) MS *m/z* (rel int) 494 (M, 2), 403 (10), 314 (3), 313 (3), 312 (3), 269 (5), 151 (8), 91 (100) ^{1}H NMR ($CDCl_3$) δ 3 8 (*s*, OMe), 3 86 (*s*, OMe), 4 98 (2H, *s*, $ArCH_2$), 5 09 (2H, *s*, $ArCH_2$), 6 65–7 35 (15H, *m*, ArH), 7 86 (1H, *s*, H-2)

2′,5′-*Dibenzyloxy*-4′-*methoxybenzyl*-4-*methoxy*-2-*hydroxyphenylketone* (**10**) A 10% soln of KOH (60 ml) was added to a stirred suspension of **9** (1 68 g) in hot EtOH (120 ml) The mixture was refluxed for 1½ hr, cooled, concd *in vacuo* and acidified with dilute HCl Extraction with EtOAc gave **10** (1 29 g), mp ($CHCl_3$–MeOH) 126–127° Found, M 484 1873, $C_{30}H_{28}O_6$ requires 484 1884 MS *m/z* (rel int) 484 (M, 2), 391 (2), 179 (35), 151 (28), 91 (100) ^{1}H NMR ($CDCl_3$) δ 3 81 (6H, *s*, OMe), 4 09 (2H, *s*, $COCH_2$), 5 02 (4H, *s*, $ArCH_2$), 6 36–7 77 (15H, *m*, ArH)

Hydrogenation of the phenylketone **10** (150 mg) over Pd–C catalyst (10%, 150 mg) in EtOH (150 ml) containing 1% HCl yielded sainfuran, **1** (48 mg), which was obtained as crystals after chromatography on silica gel with CH_2Cl_2 The synthetic sainfuran was identical (mp, mmp, MS, ^{1}H NMR, TLC) to the natural product

2′,5′-*Dibenzyloxy*-4′-*methoxybenzyl*-2,4-*dimethoxyphenylketone* (**11**) A suspension of **10** (173 mg) and K_2CO_3 (1 8 g) in DMF (17 ml) was stirred with Me_2SO_4 (50 mg) for 1½ hr at 60° Chromatography on silica gel with CH_2Cl_2 gave **11** (90 mg), mp 78–82° MS *m/z* (rel int) 498 (M, 3), 347 (3), 179 (30), 165 (73), 91 (100), 65 (15)

Hydrogenation of the phenylketone **11** (66 mg) over Pd–C catalyst (60 mg) in EtOH (60 ml) containing 1% HCl gave methylsainfuran, **2** (10 mg) after chromatography on silica gel with CH_2Cl_2 The synthetic methylsainfuran was identical (mp, mmp, MS, ^{1}H NMR, TLC) to the natural product

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