Isoceroptene, a Novel Polyphenol from *Pityrogramma triangularis*

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Isoceroptene, a polyphenol isolated in trace amount from the farinose frond exudate of *Pityrogramma triangularis,* is shown by spectroscopic means to possess the structure of one of the two possible "flavanone" isomers formed by ring closure of the "chalcone" ceroptene. The tautomeric structures best representing ceroptene and a related dihydrochalcone from *Myrica gale* are defined by ^{1}H-NMR spectroscopy.

Introduction

The flavonoids that constitute the farinose frond exudate of the Californian goldback fern, *Pityrogramma triangularis* (Kaulf.) Maxon have been studied extensively in recent years (see ref. [1]). In a recent paper [2], a total of over twenty flavonoids is reported, ten of which are new natural products. In the present communication an as yet unidentified trace constituent is examined. This constituent was of interest as it appeared to be related to the highly unusual "chalcone" ceroptene (**1**), which is the major farina component of a distinct chemotype of *P. triangularis* var. *triangularis*.

Materials and Methods

*Isolation and spectroscopy of isoceroptene (**2**)*

The plant material of *P. triangularis* var. *triangularis* (ceroptene type) is the same as reported in ref. [1], collected at various localities in California by D. M. Smith (*cf.* [5]). The frond exudate was recovered and worked up as described previously [1]. Some minor fractions with ceroptene as the major component yielded a few mg of crystalline material. The crystals consisted of compound **2** with a similar quantity of 3,5-diOH-7-OCH_3-8-CH_3-flavone and a lesser amount of 3,5-diOH-7-OCH_3-6,8-diCH_3-flavone [2]. **2** was finally obtained pure by CC on a small polyamide column.

Compound **2** forms colourless crystals, m.p. 218–220°; λ_{max}(MeOH) 335, 268, 237, 220; (+$AlCl_3$) 340, 250, 217; (+NaOH) 295, 234, 215 nm. MS *m/z* (rel. int.): 298 (100, M^+), 297 (49), 283 (18, M-15), 280 (16, M-18), 227 (23), 221 (61, M-77) 195 (38), 131 (25), 126 (40), 115 (11), 111 (22), 103 (25), 77 (20). The MS fragmentation is almost identical with that for ceroptene, **1**, except for some differences in relative intensities. ^{1}H-NMR δ (DMSO-d_6): *Isoceroptene* (**2**); 17.96 (s, 1H, 5-OH), 7.18 (s, 5H, B-ring), 5.46 (s, 1H, H-8), 4.97 (m, 2H, H-2/H-3), 3.82 (s, 3H, OCH_3), 1.27 (s, 3H, CH_3), 1.10 (s, 3H, CH_3). *Ceroptene* (**1**); 8.23, 7.90 (doublets, J = 16 Hz, 2H, H-α/H-β), 7.72, 7.48 (multiplets, 5H, B-ring), 5.69 (s, 1H, H-5′), 3.86 (s, 3H, OCH_3), 1.33 (s, 6H, CH_3). ^{1}H-NMR δ ($CDCl_3$): *Isoceroptene* (**2**); 18.28 (s, 1H, 5-OH), 7.19 (m, B-ring + $CHCl_3$), 5.14/5.00 (m, 3H, H-2,3,8), 3.64 (s, 3H, OCH_3), 1.18 (s, 3H, CH_3), 0.99 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d^6): *Isoceroptene* (**2**); 195.1/187.4 (C = 0), 139.7/128.0/127.8/126.5 (B-ring), 92.9 (C-8), 57.1 (OCH_3), 51.4 (C-2)*, 48.5 (C-6)*, 40.6 (C-3)*, 25.3/23.9 (CH_3) ppm; *Ceroptene* (**1**); 187.1 (C = 0), 144.0 (C-β), 134.8 (C-1), 130.9/129.3/128.7 (C-2, 3, 4, 5, 6), 123.3 (C-α), 95.7 (C-5′), 57.3 (OCH_3), 24.6 (CH_3) ppm, and in addition, low intensity signals were present at 196, 190, 179, 105 (C-1′) and 48.4 (C-3′) ppm (as observed previously [3]).

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Thin-layer chromatography

TLC was on polyamide (solvent A, petrol (100–140°)/toluene/MeCOEt/MeOH 90:30:2:1.5) and on silica (solvent B, toluene/MeCOEt 9:1; solvent C, toluene/petrol (100–140°)/MeCOEt 15:4:1). On polyamide the R_f of **2** is only slightly higher (0.78) than that of ceroptene (0.73) and thus is difficult to detect in crude extracts. It also may be concealed by the co-occurring 5-OH-7-OMe-6-CH_3-flavanone and 5-OH-7-OMe-8-CH_3-flavanone [2]. The spot of **2** is dark gray in UV_{366} and remains dark/absorbing on spraying with "Naturstoffreagenz A", while the black spot of **1** turns very dark brown. In UV_{254} the two spots also appear slightly different. On silica, **2** has R_f 0.56 (solv. B) and 0.25 (solv. C), **1** has 0.65 (solv. B) and 0.42 (solv. C). The best separation therefore is on silica with solvent C. The two flavanones mentioned do not interfere in this system.

*Isomerisation of ceroptene (**1**)*

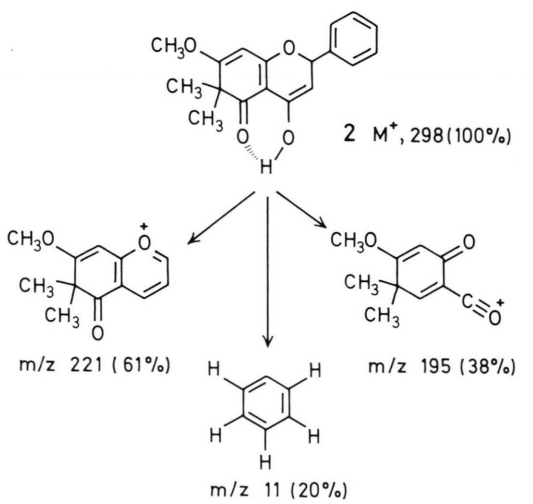
According to the method of Subrahmanyam *et al.* [4], to crystalline ceroptene (0.4 mg), in benzene (1 ml) was added TLC grade silica (MN G-HR) (0.2 g). This mixture was left for 24 hrs at RT in a flask loosely plugged with glass wool. The dry silica remaining was extracted with acetone and the extract analysed by TLC (Merck Kieselgel $60F_{254}$) in a variety of solvents, together with ceroptene and isoceroptene. The best solvent was $CHCl_3$ which resolved the extract into three components, ceroptene R_f 0.68, isoceroptene R_f 0.53, and one other R_f 0.55. Other solvents used included toluene/acetone, 19:1; $CHCl_3$/acetone 19:1 and EtOAc/$CHCl_3$, 5:95.

Results and Discussion

The new component, **2**, was found only in representatives of the "ceroptene-type" [5] of *Pityrogramma triangularis* var. *triangularis*. In the farinose frond exudate of such ferns it co-occurs with ceroptene (the major component) and a variety of further methylated, mostly C-methylated, flavonoids [2].

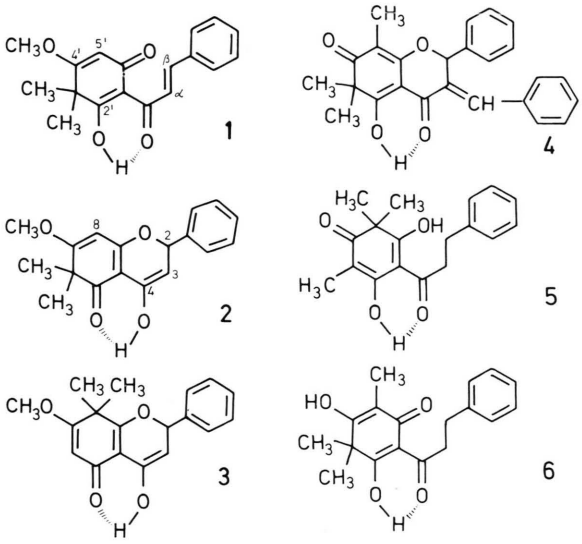
The new component, **2**, from *P. triangularis* was isolated in very small quantities as colourless crystals, m.p. 218–220°. With a M.W. (MS) of 298 it is isomeric with the co-occurring yellow pigment ceroptene (**1**). It is therefore referred to as isoceroptene.

Isoceroptene is clearly distinguished from ceroptene by its colour, its TLC mobility and by its UV-visible absorption spectrum. The absorption spectrum, while unlike the chalcone-type spectrum of ceroptene (λ 234, 300 sh, 364 nm) is also not that of a flavanone [6] thus excluding the 6,8-di-C-methyl-5-hydroxy-7-methoxy-flavanone structure. Signals in the ^{13}C-NMR spectrum indicate that in common with ceroptene, isoceroptene contains one methoxyl, one protonated A-ring carbon (92.9 ppm), one H-bonded carbonyl and a gem-dimethyl functionality. The B-ring carbon resonances appear at 139.7, 128.0, 127.8 and 126.5 ppm, chemical shifts which are typical of an unsubstituted B-ring attached to a *saturated* carbon [7] (*cf.* ceroptene). This is confirmed by the MS (Fig. 1) in which intense ions appear at *m/e* 221 (representing loss of an unsubstituted B-ring from a saturated carbon [8]) and *m/e* 77 (the unsubstituted B-ring fragment). The RDA-type fragment at *m/e* 195 derived from the A-ring indicates that the methoxyl, the gem-dimethyl, the ring proton and two oxygen containing functions, reside in the A-ring.



The structure of isoceroptene is clearly very similar to that of ceroptene, but the ^{1}H-NMR spectrum in particular suggests that isoceroptene may contain a C-ring. In isoceroptene the "equivalents" of the α- and β-protons of ceroptene (δ 7–8 region) have shifted markedly upfield. They appear as a two-proton multiplet centred at δ 4.97, a chemical shift close to that expected for H-2 of a flavanone [9] and exactly that calculated from additivity data [10] for H-3 in **2**. Structure **2** thus appears to be tenable and could be envisaged as being formed from ceroptene by a

Michael addition of the A-ring hydroxyl across the exocyclic α, β-double bond in the same manner as chalcones can be converted to flavanones [4]. An established method for carrying out this conversion is prolonged treatment of the chalcone in benzene with SiO_2 [4]. When ceroptene was treated in this way, a partial conversion to isoceroptene was achieved thereby confirming a flavenol-type structure for isoceroptene. Because of the keto-enol tautomerism possible in a structure like **1**, two different ring closures are possible, one of which would lead to **2** and the other to its isomer **3**. Indeed, analysis of the products of Bz/SiO_2 ring closure of ceroptene revealed, in addition to unchanged ceroptene, two very closely related products one of which was identical to isoceroptene. Similar analysis of natural isoceroptene revealed the presence of only one of these isomers, thereby confirming that isoceroptene is indeed a natural product and not an artifact produced from ceroptene during the isolation process.



Of the two possible structures, **2** and **3**, it is considered that **2** best represents isoceroptene. Examination of Dreiding models reveals that in structure **3** the methyl groups of the gem-dimethyl function are in similar environments while in structure **2** they are in very different environments. In **2**, one methyl is virtually in the plane of the A-ring carbonyl while the other is at about 120° to this plane. Thus, in **2** the methyl resonances would be expected to appear as two three-proton singlets due to the neighbouring anisotropic effect of the carbonyl. The spectrum of isoceroptene does in fact contain two three-proton singlets. These are observed for both $CDCl_3$ and DMSO-d_6 solutions, thereby excluding the possibility that the chemical shift differences are due to solvent effects such as have been observed in steroids containing "4,4-dimethyl-3-one" functionalities [11]. Accordingly isoceroptene is assigned the structure **2**. Two three-proton singlets have also been observed for the gem-dimethyl functionality in **4** [12], a compound which is structurally analogous to isoceroptene. The chemical shift difference between these two singlets was reported as 0.16 ppm, a figure which compares well with that observed for isoceroptene (0.17, DMSO; 0.19, $CDCl_3$). In ceroptene, on the other hand, the gem-dimethyl group appears as a six-proton singlet which confirms the structure **1** for ceroptene (in DMSO) rather than the alternative isomer with the gem-dimethyl adjacent to the A-ring carbonyl.

On the basis of the type of reasoning used above it would seem that the structure of the "dihydrochalcone" **5**, which was isolated from *Myrica gale* [13], is in need of modification. In the reported ^{1}H-NMR spectra of **5** [12, 13] and its triacetate [14] the gem-dimethyl protons appear as six-proton singlets at 1.33 and 1.26 respectively. This indicates that the carbonyl is not adjacent to the gem-dimethyl grouping. Structure **6** is therefore favoured over structure **5** which was proposed.

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