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FLAVONOIDS FROM *TEPHROSIA FULVINERVIS*

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Abstract—From the pods of *Tephrosia fulvinervis*, a new Ethiopian species, a new flavanone, fulvinervin A, and a new flavone, fulvinervin B, have been isolated and their structures elucidated from their chemical properties and spectral data.

INTRODUCTION

The genus *Tephrosia* elaborates several types of compounds whose chemotaxonomic importance in the genus has been discussed [1]. In continuation of our studies on *Tephrosia* species we report here the examination of *T. fulvinervis*, a species known only from Ethiopia, which has apparently not been investigated previously. The chloroform extract of pods on CC on silica gel yielded a new flavanone and a new flavone designated fulvinervin A (**1**) and fulvinervin B (**4**), respectively.

RESULTS AND DISCUSSION

Both fulvinervin A (**1**) and fulvinervin B (**4**) gave positive tests with ferric chloride (green) and lead acetate (yellow precipitate), but a negative Shinoda test. Both exhibited a pale brown fluorescence which intensified with ammonia vapour in UV light. Both also gave a positive test with Wilson's boric–citric acid reagent [2] (yellow) indicating the presence of a 5-hydroxyl or a 5-methoxyl but ^{1}H NMR showed a chelated hydroxyl in off-set, with no peak for supporting the presence of a 5-methoxyl.

Fulvinervin A (**1**), mp 128–130°, showed a $[M]^+$ m/z 390 consistent with the formula $C_{25}H_{26}O_4$. The ^{1}H NMR spectrum showed the resonances typical of the ABX spin system of the flavanone saturated ring [3, 4], which are clearly resolved into an AMX system at 300 MHz, allowing first order analysis [5]: δ5.42 (1H, *dd*, $J_{\text{H-2}_{ax}/\text{H-3}_{ax}}$ = 12.9 Hz, $J_{\text{H-2}_{ax}/\text{H-3}_{eq}}$ = 3.2 Hz, H-2_{ax}), 3.06 (1H, *dd*, $J_{\text{H-3}_{ax}/\text{H-3}_{eq}}$ = 17.1 Hz, $J_{\text{H-3}_{ax}/\text{H-2}_{ax}}$ = 12.9 Hz, H-3_{ax}), 2.84 (1H, *dd*, $J_{\text{H-3}_{eq}/\text{H-3}_{ax}}$ = 17.1 Hz, $J_{\text{H-3}_{eq}/\text{H-2}_{ax}}$ = 3.2 Hz, H-3_{eq}), which indicates that the conformation of the C-ring is half chair with that of the 2-phenyl being equatorial. The spectrum also showed a set of peaks, characteristic of a 2,2-dimethylchromene ring [6], and another set characteristic of a C-3-methylbut-2-enyl (= γ,γ-dimethyl allyl = prenyl) group [5, 7]. The aromatic region exhibited no A-ring proton but only a complex and narrow multiplet attributed to the unsubstituted unconjugated B-ring of a flavanone [4, 8]. The spectrum also revealed a chelated phenolic hydroxyl at δ12.38 (1H, *s*, OH-5). These data support the isomeric structures of either **1** or **2**.

In the mass spectrum of fulvinervin A (**1**) a loss of 56 mass units was observed, similar to fleminone [5], supporting the angular structure. The mass fragmentation pattern was identical to that reported for fleminone [5] except for the B-ring part. The IR spectrum of fulvinervin A (**1**) showed no hydroxyl band, but a keto group at 1620 cm^{-1} (chelated) indicated that the 5-hydroxyl may be chelated as in pongaglabrol [9]. Cyclization yielded a chroman derivative [10] (**3**), mp 169–172°, ferric negative and IR of this derivative showed a shift of the carbonyl to 1680 cm^{-1} showing the involvement of the 5-hydroxyl in chelation. Furthermore, in the UV spectrum, this compound showed no shift with aluminimum chloride–hydrochloric acid due to steric hindrance [11] like fleminone, thus confirming the assigned structure (**1**) for fulvinervin A. The high negative ORD value, $[\alpha]_D$ −74.38°, suggests that the flavanone has the 2*S* configuration at C-2. Moreover, the CD spectrum of fulvinervin A exhibited a positive Cotton effect due to the $n \rightarrow \pi^*$ transition (~ 320–330 nm) and a negative Cotton effect in the $\pi \rightarrow \pi^*$ region (~ 270–310 nm) with the 2-aryl group substituted equatorially to the heterocyclic ring [5, 12].

The reported flavonoids cajaflavanone [7] and fleminone [5] differ from fulvinervin A in having substituents on ring B.

Fulvinervin B (**4**), mp 172–174°, showed a $[M]^+$ m/z 386 consistent with the formula $C_{25}H_{22}O_4$. The ^{1}H NMR spectrum showed the typical heterocyclic proton of an unsaturated C-ring which is observed at low field: δ6.71 (1H, *s*, H-3) and is characteristic of a flavone [3, 4, 8]. As in fulvinervin A, no A-ring proton was observed but the aromatic region showed two well separated complex multiplets diagnostic of an unsubstituted conjugated B-ring of a flavone [4, 8]. It also revealed a set of peaks characteristic of a 2,2-dimethylchromene [6]. It showed another typical set of peaks characteristic of the unusual γ-methyl-*trans*-but-α,γ-dienyl side chain [13–15]: δ7.38 (1H, *d*, J = 16.5 Hz, H-β), 6.86 (1H, *d*, J = 16.5 Hz, H-α), 5.11, 5.12 (2H, 2*s*, CH_2-δ), 2.08 (3H, *s*, Me-γ). A signal was

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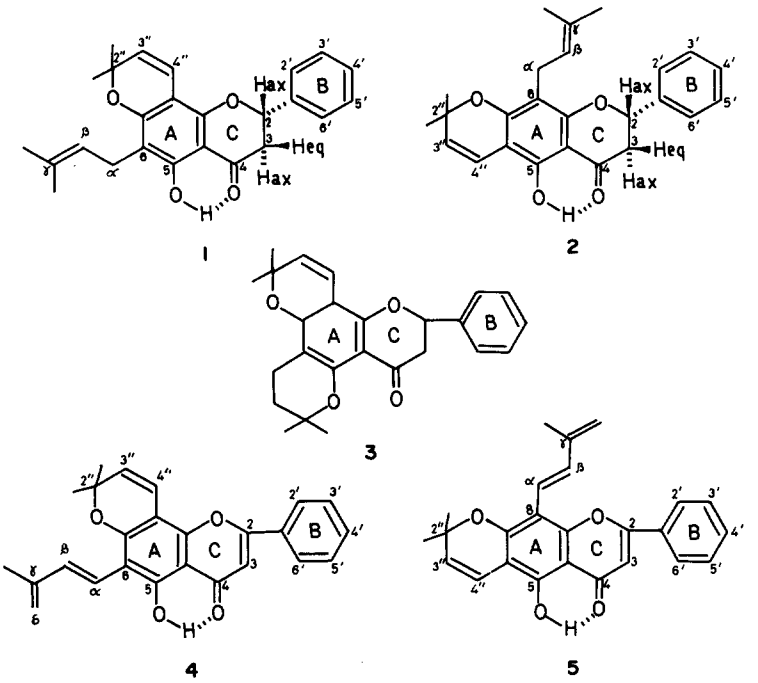


also observed in off set at δ13.28 [1H, *s*, OH-5 (chelated)]. As in fulvinervin A, these data support the isomeric structures of either **4** or **5**. The IR spectrum showed a carbonyl band at 1620 cm^{-1} (chelated) and no hydroxyl band as observed in fulvinervin A.

The mass spectrum (Scheme 1) showed the loss of a methyl to give the pyrilium cation *m/z* 371, which is the base peak of the spectrum. This cation lost water which could only be released by cyclization between the γ-methyl-*trans*-but-α,γ-dienyl side chain and an adjacent hydroxyl, resulting in the proposed fragment *m/z* 353 having a new benzene ring. The $[M]^+$ also showed the loss of 41 mass units (C_3H_5), giving the fragment *m/z* 345. This fragmentation has already been observed for the flavanone (−)-dehydroisoderricin which has the same side chain at C-8, but with an adjacent methoxyl group [15]. However, the loss of 42 mass units (C_3H_6), observed in the mass spectrum of fulvinervin B (**4**) from *m/z* 371 to *m/z* 329, was not observed for (−)-dehydroisoderricin and probably results from cyclization between the side chain and an *ortho*-hydroxyl. Hence, the loss of 42 mass units appears to be a decisive criterion for the structural assignment of a γ-methyl-*trans*-but-α-γ-dienyl chain adjacent to a 5-hydroxyl. Furthermore, the fragment at *m/z* 369, probably resulting from the loss of H_2 from *m/z* 371, could have the structure shown in Scheme 1, with a new ring formed again between the side chain and the adjacent hydroxyl. All these data support the angular structure **4** and exclude the alternative structure **5**.

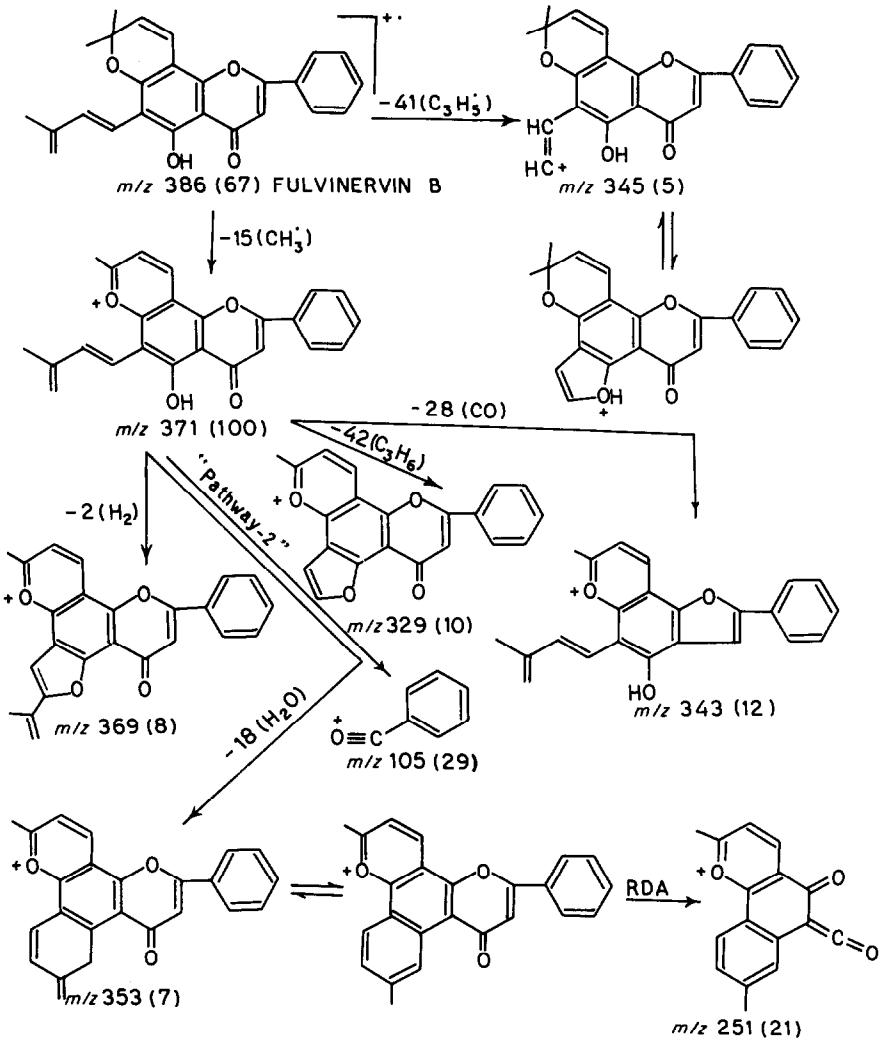
In the UV spectrum of fulvinervin B (**4**), the aluminium chloride–hydrochloric acid induced shift of the shorter wavelength band is only +3 nm due to steric hindrance [11] caused by the adjacent isoprenoid substituent. This further confirms the assigned structure **4** for fulvinervin B.

EXPERIMENTAL

Plant material was collected near Jimma in Ethiopia in August 1982. Air dried pods (600 g) were extracted with petrol, $CHCl_3$ and EtOH. Concn of the $CHCl_3$ extract yielded 7 g of a greenish syrup. This was chromatographed on Si gel (ACME, finer than 200 mesh) and eluted with petrol, C_6H_6 and $CHCl_3$ in increasing order of polarity. Fulvinervin A was obtained in petrol–C_6H_6 (3:1) fractions as pale yellow needles (60 mg) and fulvinervin B in C_6H_6 fractions as deep yellow needles (25 mg).

Fulvinervin A. Mp 128–130°, UV λ_{max}^{MeOH} nm (log ε): 229 sh (4.01), 266 sh (4.39), 272 (4.40), 299 (3.87), 311 (3.84), 365 (3.47). No changes were observed after the addition of shift reagents. IR ν_{max}^{KBr} cm^{-1}: 2890, 1620 (ArC=O), 1590, 1560, 1530 (ArH), 1380 and 1400 (gem-Me_2C). ^1H NMR (300 MHz, $CDCl_3$): δ5.42 (1H, *dd*, H-2_{ax}), 3.06 (1H, *dd*, H-3_{ax}), 2.84 (1H, *dd*, H-3_{eq}), 7.41–7.49 (5H, *m*, H-2′–H-6′), 6.59 (1H, *d*, J = 10 Hz, H-4″), 5.49 (1H, *d*, J = 10 Hz, H-3″), 1.48 (3H, *s*), 1.45 (3H, *s*, 2Me-2″), 5.24 (1H, *t*, J = 7.2 Hz, H-β), 3.28 (2H, *d*, J = 7.2 Hz, CH_2-α), 1.82 (3H, *s*), 1.71 (3H, *s*, 2Me-γ), 12.38 (1H, *s*, OH-5). MS *m/z* (rel. int.): 391 (12), (390) (36) $[M]^+$, 388 (7) $[M-H_2]^+$, 376 (24), 375 (87) $[M-Me]^+$, 373 (11), 347 (9), 335 (14) $[M-C_4H_7]^+$, 319 (21), 271 (28), 269 (13), 243 (18), 231 (17), 215 (100), 77 (21). $[\alpha]_D^{25}$ −74.38 ($CHCl_3$; *c* 0.65). CD (1.6 mg in 10 ml $CHCl_3$) $\Delta\varepsilon_{\lambda(nm)}$: $\Delta\varepsilon_{333}$ 0, $\Delta\varepsilon_{322}$ +1.46, $\Delta\varepsilon_{318}$ 0, $\Delta\varepsilon_{311}$ −3.00, $\Delta\varepsilon_{301}$ −4.47, $\Delta\varepsilon_{286}$ −1.87, $\Delta\varepsilon_{280}$ −3.09, $\Delta\varepsilon_{278}$ −2.60, $\Delta\varepsilon_{276}$ −2.84, $\Delta\varepsilon_{250}$ 0.

Cyclization of fulvinervin A to chroman (**3**). Fulvinervin A (10 mg) was taken up in 2 ml HCO_2H and a few drops of conc. H_2SO_4 were added. The mixture was warmed for a few minutes whilst shaking until dissolved. The clear acid soln was allowed to stand at room temp. for 24 hr. The material was then poured over crushed ice and gently shaken. The material was filtered, washed, dried and crystallized from petrol–C_6H_6, mp 169–172°; ferric



Scheme 1. Mass spectral fragmentation of fulvinervin B (4).

negative, IR ν_{max}^{KBr} cm^{-1}: 2980, 1680 (ArC=O), 1590, 1570 (ArH), 1390, 1400 (gem-Me_2C).

Fulvinervin B. Mp 172–174°, UV λ_{max}^{MeOH} nm (log ε): 218 (4.28), 280 (4.53), 295 sh (4.50), 309 sh (4.42), 355 sh (3.57). IR ν_{max}^{KBr} cm^{-1}: 3030, 2960, 1620 (ArC=O), 1580, 1560, 1530 (ArH), 1375, 1385 (gem-Me_2C). ^1H NMR (300 MHz, $CDCl_3$): δ6.71 (1H, *s*, H-3), 7.92–7.95 (2H, *m*, H-2′, H-6′), 7.54–7.58 (3H, *m*, H-3′–H-5′), 6.77 (1H, *d*, J = 10 Hz, H-4″), 5.68 (1H, *d*, J = 10 Hz, H-3″), 1.55 (6H, *s*, 2Me-2″), 7.38 (1H, *d*, J = 16.5 Hz, H-β), 6.86 (1H, *d*, J = 16.5 Hz, H-α), 5.11, 5.12 (2H, 2*s*, CH_2-δ), 2.08 (3H, *s*, Me-γ), 13.28 (1H, *s*, OH-5). MS *m/z* (rel. int.): 387 (19), 386 (67) $[M]^+$, 385 (7), 372 (26), 371 (100) $[M-Me]^+$, 369 (8), 353 (7), 345 (5) $[M-C_3H_5]^+$, 343 (12), 329 (10), 315 (6), 303 (5), 251 (21), 105 (29) $[C_6H_5C{\equiv}O]^+$, 77 (21).

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