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FLAVONOIDS FROM CHEMOTYPES OF THE GOLDBACK FERN, *PITYROGRAMMA TRIANGULARIS*

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Abstract—More than 20 flavonoid aglycones were identified from the frond exudate of various collections of *Pityrogramma triangularis* Ten of them are novel natural products, most of the others are rare flavonoids They are substituted at C-8, many are *C*-methylated They mostly occur only as trace constituents in var *triangularis* Two compounds come from var *viscosa* One population of *P triangularis* var *triangularis* exhibits a unique flavonoid pattern not encountered before in any other chemotype studied

INTRODUCTION

The conspicuous yellow coating on the under frond surface of the California Goldback Fern, *Pityrogramma triangularis* (Kaulf) Maxon, was first analysed by Blasdale [1, 2] at the turn of the century He isolated the major constituent of this exudate, which he called 'ceroptene' (*cera* = Latin for wax, *ptene* probably modified from Greek *pteris*, *pteridos* = fern) and recognized it as a benzene derivative It was not until half a century later that Nilsson [3] established the structural formula of this natural product as a chalcone-like substance (**1**) Within the *P triangularis* var *triangularis* group [4], plants producing ceroptene represent the 'ceroptene-type', to which also the holotype of the species belongs [5] From the same chemotype, two further flavonoids were reported later, namely pityrogrammin (3,5,7-trihydroxy-8-methoxy-6-*C*-methylflavone) [6] and triangularin (2',6',4-trihydroxy-4'-methoxy-3'-*C*-methylchalcone) [7] (**2, 17**) Dietz *et al* [8] reported 5,7-dihydroxy-3-methoxy-6,8-di-*C*-methylflavone (**14**) from the ceroptene-type However, as Smith noted [9] a significant number of minor components of the exudate still remained unidentified We have now been able to analyse and identify a series of compounds from material of the *P triangularis* var *triangularis* group, derived from different populations As it turned out, one of the flavones reported earlier [8] needs revision Also, two new flavonols were identified from *P triangularis* var *viscosa* in addition to the previously described *C*-methyldihydrochalcone (**3**) [10] Thus in the present paper we report more than 20 flavonoids, most of which are new for *P triangularis* (Figs 1 and 2) Ten of them are novel natural products

RESULTS

Spectral data are presented below for the rare and novel flavonoids, along with their occurrence in the different collections or populations

Compound **4** was reported previously to be 5,7-dihydroxy-6,8-dimethoxyflavone (**2** in ref [8]) However, the spectral data had been misinterpreted, i e one methoxyl group had been placed at C-8 instead of C-7 and the second had been erroneously placed at C-6 instead of at C-3 Therefore, **4** is 5,8-dihydroxy-3,7-dimethoxyflavone, the 3,7-dimethyl ether of 8-hydroxygalangin (isognaphalin) Its identity was unambiguously proved by direct comparison (TLC, UV) with a synthetic sample [11] The mps of **4** (222–223°) and its acetate (232–234°) are in accord with literature values (216–218° for the flavone, 231–234° for its acetate [11]) The mass spectral fragmentation of **4** agreed with that of several other flavonols with the same substitution pattern (F J Arriaga, personal communication) Further, its possible identity as 8-hydroxygalangin 3,8-dimethyl ether, 8-hydroxygalangin 7,8-dimethyl ether or 6-hydroxygalangin 3,6-dimethyl ether was excluded by direct comparison with markers The erroneous structure suggested for **4** in ref [8] also needs to be cancelled in ref [12] (p 192)

Only a very small, non-crystalline and impure quantity of compound **5** could be isolated The $[M]^+$ at *m/z* 330 indicated a flavone with three hydroxyl groups and two methoxyl groups, as confirmed by the appropriate signals in the ^1H NMR spectrum The UV spectrum showed that there were hydroxyl groups at C-5, C-7 and C-4', no *O*-dihydroxyl group, no methyl or methoxyl at C-6 The colour behaviour on polyamide pointed to an 8-*O*-substituted compound This led to the structure of 5,7,4'-trihydroxy-3,8-dimethoxyflavone Direct comparison (TLC, UV) with a synthetic sample or herbacetin 3,8-dimethyl ether indeed confirmed the identity of **5** with this product

Compound **6** showed on polyamide TLC the same colour behaviour as herbacetin 8-methyl ether (dark orange spot under UV 366 turning yellow-brown with NA reagent) and the difference in R_f indicated methylation of one hydroxyl group The $[M]^+$ was at *m/z* 330 again, indicative of a flavone with three hydroxyl groups and two

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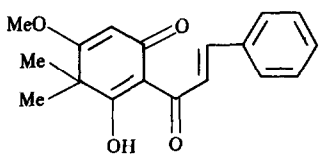
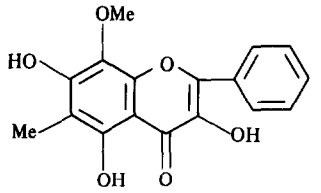
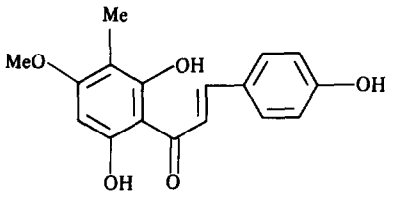


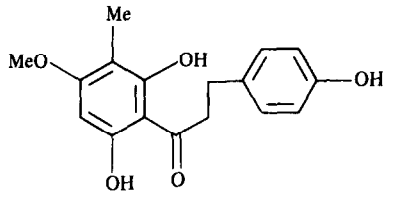
1 Ceroptene



2 Pityrogrammin



17 Triangularin



3 2,6,4 – OH, 4 – OMe, 3 – Me – dihydrochalcone

Fig 1 Structural formulae of previously known flavonoids from *Pityrogramma triangularis*

methoxyl groups The base peak appeared at $[M-15]^+$, thus indicating a methoxyl at C-8 (cf ref [12], p 243) The UV spectral data were also in favour of an 8-substituted flavone (three maxima in methanol, cf ref [12], p 241) and allowed the second methoxyl to be placed at C-4' Direct comparison with an authentic sample of herbacetin 8,4'-dimethyl ether [13] showed that **6** was identical with prudomestin

Compound **7** formed yellow needles, but the amount was too small for a mp determination Again the UV and mass spectra suggested an 8-methoxyflavone, the $[M]^+$ and ^1H NMR signals indicated the presence of a 3-methoxyl group Evaluation of TLC behaviour and complete interpretation of spectral data led to the conclusion that this product must be the 3-methyl ether of **6** and the spectral data were in accordance with those reported in the literature for herbacetin 3,8,4'-trimethyl ether [14, 15]

Compound **8** appeared on polyamide as a dark spot that turned greenish-yellow with NA reagent The $[M]^+$ at m/z 284 allowed a flavone with either two hydroxyl and one methoxyl group or three hydroxyl and one methyl group Important fragments at m/z 105 and 77 pointed to an unsubstituted B-ring According to the R_f, the *C*-methylated product is more likely and this assumption was confirmed by the presence of a methyl signal in the ^1H NMR spectrum Since the second peak in band I (MeOH) pointed to C-8 substitution and the shift reagents revealed free hydroxyl groups at C-3, C-5 and C-7, the methyl group should be placed at C-8 **8** is therefore 3,5,7-trihydroxy-8-methylflavone (8-*C*-methylgalangin)

Compound **9** was obtained by preparative TLC on silica and purified on Sephadex LH-20 It formed a dark spot that turned brown with NA The $[M]^+$ at m/z 298 allowed a flavone with either one hydroxyl and two methoxyl groups or two hydroxyl, one methoxyl and one methyl group The ^1H NMR signals showed that the latter was true Again the B-ring was unsubstituted Further interpretation of the spectral data compared with the previously published structure of **14** [8] showed **9** to be either desmethyl-**14** or 3-methyl-**8**, i e its structure is 5,7-dihydroxy-3-methoxy-8-methylflavone

Compound **10** was obtained as yellow needles, mp 197° Its acetate had mp 187–188° **10** also showed the properties of a flavone with two hydroxyl, one methoxyl and one methyl group, an unsubstituted B-ring and C-8 substitution Like **8**, it appeared on TLC as a dark spot that turned greenish-yellow with NA A ^{13}C NMR spectrum was run and its interpretation according to refs [16–19] is shown in Fig 3 From all the spectral data, **10** is thus identified as 3,5-dihydroxy-7-methoxy-8-methylflavone The unambiguous structure elucidation of this compound also allowed valuable conclusions concerning the structures of **8** and **9**

Compound **11** was isolated only in trace amount It appeared dull greenish-brown on polyamide, becoming dark with NA and appeared reddish-brown in daylight The R_f, mass and ^1H NMR spectra revealed that **11** was a flavone with three hydroxyl, one methoxyl and one methyl group, an unsubstituted B-ring and a completely substituted A-ring The positions of the substituents were ascribed according to the spectral data and **11** was found to be 3,5,8-trihydroxy-7-methoxy-6-methylflavone This structure is isomeric with that reported previously [6] for pityrogrammin (3,5,7-trihydroxy-8-methoxy-6-methylflavone) (**2**) from the same chemotype of *P triangularis* var *triangularis* Unfortunately an authentic sample of this flavonoid was not available for direct comparison The spectral data indicated, however, that both products were indeed different substances

Compound **12** also showed no NA reaction in UV, but the same reddish colour in daylight as **11** The R_f difference and $[M]^+$ indicated that it was a methyl derivative of **11** The spectra indicated placement of the additional methyl group at C-3 **12** is thus 5,8-dihydroxy-3,7-dimethoxy-6-methylflavone **11** could not be sep-

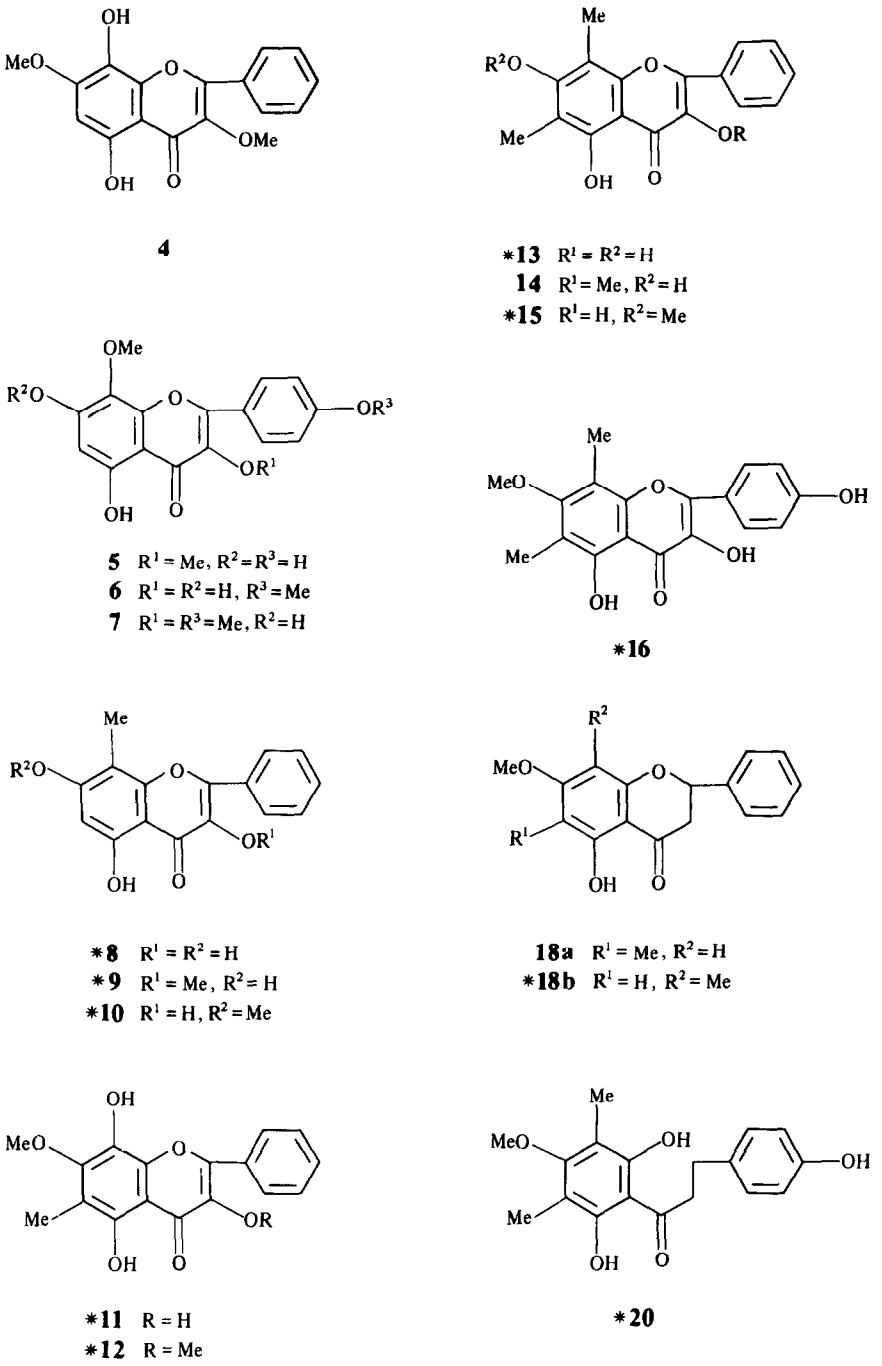


Fig 2 Structural formulae of the flavonoids now found in *Pityrogramma triangularis* Novel compounds are marked with an asterisk

arated from **4** on polyamide, whereas on silica they had clearly different R_f values (**4** 0 26, **12** 0 37, solvent D)

Compound **13** appeared as a dark spot on TLC that turned dull yellow with NA The $[M]^+$ was at *m/z* 298, like **10**, but the ^1H NMR spectrum showed that the substituents were three hydroxyl and two methyl groups According to UV reactions with classical shift reagents, there were free hydroxyl groups at C-3 and C-7 Since mass spectral fragmentation showed an unsubstituted B-ring, the third hydroxyl was placed at C-5 and the two methyls were at C-6 and C-8, respectively **13** is thus 3,5,7-trihydroxy-6,8-dimethylflavone

Compound **14** was reported previously (**1** in ref [8]). It is 5,7-dihydroxy-3-methoxy-6,8-dimethylflavone

Compound **15** showed the same colour behaviour on TLC as **13**, but at considerably higher R_f, the difference suggesting methylation of the 7-hydroxyl group All the spectral data were also in favour of a 7-methyl derivative

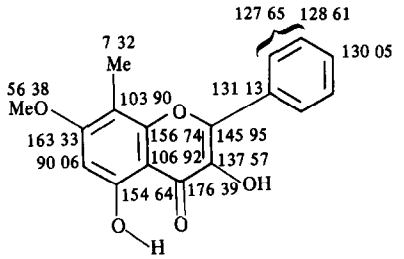


Fig 3 Structure of **10** with ^{13}C NMR values

of **13** Thus **15** is identified as 3,5-dihydroxy-7-methoxy-6,8-dimethylflavone

Compound **16** crystallized from acetone as fine yellow needles, mp 263–264°, appeared dark on polyamide, and turned reddish-brown with NA The $[M]^+$ at *m/z* 328 and the ^1H NMR signals suggested a flavone with three hydroxyl, one methoxyl and two methyl groups According to the UV-shift with sodium hydroxide, the B-ring has a free hydroxyl group at C-4′ Thus both methyl groups must be located on ring A The 5-hydroxyl group probably does not react with aluminium chloride because of steric hindrance, the shift is caused by the 3-hydroxyl group The methoxyl is located at C-7 **16** is thus identified as 3,5,4′-trihydroxy-7-methoxy-6,8-dimethylflavone (di-*C*-methylrhamnetin) The spectral data were similar to, but not identical with, those reported for the isomeric compound, 5,7,4′-trihydroxy-3-methoxy-6,8-dimethylflavone [20]

Compound **17** formed deep orange-red crystals, mp 181–183° The colour of **17** and spectral studies suggested it to be the recently described chalcone, triangularin [7] (**2**) Direct comparison with an authentic sample of 2′,6′-dihydroxy-4′-methoxy-3′-methylchalcone corroborated this result Its mp was not reported previously

When the occurrence of triangularin was reported [7], the authors mentioned that they also found 5-hydroxy-7-methoxy-6-methylflavanone as an expected cyclization product of the chalcone in all their samples of triangularin We found this flavanone too (**18a**, mp 78°), but we found it along with the corresponding 8-methylflavanone (**18b**) They are both cyclization products of the same chalcone (theoretically from 2′,6′-dihydroxy-4′-methoxy-5′-methylchalcone) In fact, heating a sample of triangularin above its mp yields a mixture of both flavanones This corresponds to a reaction observed previously for the chalcone pashanone, produced by the fern *Onychium siliculosum* [21] In their chromatographic behaviour the flavanones **18a** and **18b** correspond to the behaviour of those found in *Onychium* and also parallel the behaviour of other flavanone pairs (**2**/**4** and **1**/**3** in ref [22]) reported as farina constituents of *Cheilanthes argentea* In every case the 6-substituted compound is dark and runs just a little bit lower (on polyamide) than the 8-substituted compound, which appears brown **18a** and **18b** could be separated by preparative TLC on silica gel (where the R_f arrangement is inverse) and their identities were corroborated by direct comparison with the methylation products (DMSO) of stroboponin (6-methyl) and cryptostrobin (8-methyl) [23] Thus **18a** is 5-hydroxy-7-methoxy-6-methylflavanone and **18b** is 5-hydroxy-7-methoxy-8-methylflavanone Like Star *et al* [7], we also assume that in the natural fern exudate the chalcone triangularin is the original constituent, whereas the flavanones **18a** and **18b** are artefacts formed during isolation (cf ref [21])

Compound **19** showed features typical for a chalcone It was readily identified by direct comparison with a synthetic sample of 6′-hydroxy-2′,4′-dimethoxychalcone (Schiemenz, G P and Klein, E , unpublished results) **19** is in fact identical with flavokawin B, which we found as a trace constituent in the farina of *P triangularis* var *pallida* [23]

Compound **20** was found to be a dihydrochalcone In several respects it resembled the earlier described *C*-methylated dihydrochalcone from *P triangularis* var *viscosa* [10] The mass and ^1H NMR spectra indicated that **20** also bears a hydroxyl group on the B-ring and the A-ring has an additional methyl group Thus **20** is 2′,6′,4-trihydroxy-4′-methoxy-3′,5′-dihydrochalcone

Several trace constituents of the fern found exudate were identified by direct comparison with markers Data are not given here for these now rather trivial flavonoids such as pinocembrin, galangin 3-methyl ether and kaempferol 3,7-dimethyl ether

One further product still awaits structural elucidation It is a colourless substance with the same $[M]^+$ and almost identical mass spectral fragmentation as ceroptene and it is found in the same chemotype It therefore could be the flavanone formed on cyclization of ceroptene

DISCUSSION

Structures

Ten flavonoids reported here are novel products These compounds, **8–16**, **18b** and **20**, are marked with an asterisk in Fig 2 To our knowledge, they have not been found earlier as plant constituents (cf refs [12, 24]) It should be stressed that 'pityrogrammin' could not be detected in any of the plant material analysed for the present work **11** and **12** indeed show the same substitution patterns as 'pityrogrammin', but not identical substitution No flavonol was known before with the substitution pattern of **8**, **9** and **10** The substitution patterns of **13** and **15** were new when we reported **14** previously [8] A compound isomeric to **16** has been isolated from the bark and spines of *Alluaudia dumosa* and *A humberti* and in *Didiera madagascariensis* [20, 25] **18b**, as discussed above, is the isomer of **18a**, which had been reported as an artefact derived from triangularin [7] Although it is expected that the two isomers occur jointly in plants producing triangularin, **18b** had not been noticed by the earlier authors These two flavanones have the same substitution patterns as the known flavanones stroboponin and cryptostrobin, respectively The substitution pattern of the dihydrochalcone **20** was new when we recently described its desmethyl analogue from the same plant material [10]

The structures of several further flavonoids have been added to Fig. 2 and their spectral properties are presented in the Experimental because they are very rare compounds in nature For the same reason, we want to cite briefly where they have been found previously **4**, the 3,7-dimethyl ether of 8-hydroxygalangin, has been found only once before, in *Achyrocline satureoides* (Asteraceae) Its structure was confirmed by synthesis [11] **5**, **6** and **7** are methyl derivatives of herbacetin (8-hydroxykaempferol) **5**, herbacetin 3,8-dimethyl ether, has also been found only

once before, namely in *Cyanostegia angustifolia* (Verbenaceae) [26] **6**, herbacetin 8,4'-dimethyl ether, was found in the heartwood of *Prunus domestica* (Rosaceae) [27] and called prudomestin Later reports concerned synthesis only [13, 28] **7**, herbacetin 3,8,4'-trimethyl ether, was known as a constituent of *Conyza stricta* (Asteraceae) [14] and *Bayeria* sp (Euphorbiaceae) [29]

All the flavonoids shown in Fig 2 bear substituents at C-8, and most of them are *C*-methylated In a previous report [10], it was mentioned that the capacity for biosynthesis of *C*-methylated flavonoids might be a typical feature of the species complex *P triangularis* within the genus *Pityrogramma* This statement should be modified insofar as it is true only for certain varieties (var *pallida* and var *viscosa*) and for certain chemotypes within a variety (ceroptene-type of var *triangularis*) With this restriction, it is nicely corroborated by the results reported here as well as by our previous report on new flavanones from var *pallida* [23]

Distribution of the compounds described

The plant material from which we isolated the flavonoids reported in the present paper comes from different sources (see Experimental) The major portion comprised plants from various origins (collected by D M Smith and M S Taylor), all clearly belonging to the ceroptene-type of *P triangularis* var *triangularis* About two-thirds of the compounds described here were isolated from this bulk collection (except for **5–7**, **16**, **19** and **20**) The material collected at Pardee Reservoir (EW-2 and EW-3) came from two populations considerably removed geographically from the range of ceroptene chemotypes and neither produces ceroptene In collection EW-3, the major farina constituent is kaempferol 4'-methyl ether It also contains traces of kaempferol and its 3-methyl ether Another important component is the flavonol **7**, and there is a smaller amount of flavonol **6**. Further flavonoids found here are the dihydrochalcone **20** and galangin 3-methyl ether Compounds **6**, **7** and **20** have so far been encountered in this population only Such plants have some chemical affinity with the widespread chemotype of the Sierra Nevada

In collection EW-2, on the other hand, the major farina component is 2',6',4-trihydroxy-4'-methoxy-3'-methyldihydrochalcone (**3**) Further dominant constituents are 2',6'-dihydroxy-4'-methoxychalcone, 2',6'-dihydroxy-4',4-dimethoxychalcone and galangin 5,7-dimethyl ether [30] Minor constituents are **4**, **12**, kaempferol 3,7-dimethyl ether, pinocembrin 7-methyl ether, and the chalcone flavokawin B (found only in this material) Galangin, galangin 3-methyl ether, galangin 7-methyl ether, kaempferol 3- and 7-methyl ethers, **18a**, **18b**, 2',6'-dihydroxy-4'-dimethoxydihydrochalcone and 2',6'-hydroxy-4',4'-dimethoxydihydrochalcone are trace constituents Galangin 5-methyl ether seems to be present too, but could not be unequivocally identified Sample EW-2 thus differs markedly from any known chemotypes of any of the named varieties of *P triangularis* Further, there is in EW-2 one constituent which, after spraying of TLC plates with NA, showed up as a bluish-violet spot in daylight, absorbing in UV Its colour reaction indicated it to be an 8-hydroxyflavonol This same unknown product is also present in material of *P triangularis* var *viscosa*, from which 2',6',4-trihydroxy-4'-methoxy-3'-methyldihydrochalcone was reported previously as the major farina constituent [30] We have now isolated from the remaining fractions the rare flavonol **5** and the novel compound **16** In addition, we found small amounts of kaempferol and its 3-methyl, 7-methyl and 3,7-dimethyl ethers

Dietz [31] has compared farina flavonoid patterns of the individual plants combined in the ceroptene-type bulk collection worked up here, as well as on further vouchers from Smith More detailed studies will be possible now that many more constituents are known

Collections EW-2 and EW-3 from Pardee Reservoir represent two smaller populations, growing at a distance of several hundred meters from each other They are both readily identifiable as *P triangularis* var *triangularis* Chemically, EW-3 is very close to the kaempferol methyl ether-chemotype of this variety as characterized by Smith [9], producing kaempferol 4'-methyl ether as the dominant farina constituent We were not able, though, to detect **6**, **7** and/or **20** in Smith's vouchers as, for example, SRP 16, SRP 30, etc [4] The amounts present might be too small and the spots concealed by others EW-3 has one unidentified constituent in common with SRP 30

The population EW-2 deserves special attention because it exhibits a very peculiar farina flavonoid pattern What is perhaps most surprising is the fact that here we find those chalcones and dihydrochalcones which are the characteristic and often the sole constituents of the farina of species like *P calomelanos*, *P chrysophylla*, *P dealbata*, *P tartarea* [32] (There were errors in the structural formulae published in ref [32], see ref [33]) The chalcones and dihydrochalcones have not been found before in any of the many samples of *P triangularis* studied so far Another characteristic feature of EW-2 is the presence of galangin 5,7-dimethyl ether, which appears as a brillant yellow spot on TLC (UV 366) [30] In total, this is a unique flavonoid pattern which imparts special interest to this population Cytological and other suitable studies should be done on these plants

We continue to check plants from many localities to see what other flavonoid patterns might be revealed For the same reason, specimens in herbaria are being checked It thus may be determined unambiguously to which chemotypes they belong and it becomes obvious when any additional flavonoid patterns are discovered Many specimens even in great herbaria are not determined to the variety This can be done readily by comparative TLC Arrangements have been made (E W) for all specimens kept at the U S National Herbarium, Washington, D C to be checked in these respects in the near future

EXPERIMENTAL

Fronds of *Pityrogramma triangularis* were collected in California The major batch consisted of several collections by D M Smith, all representing 4*n* plants of the ceroptene-type (from Hoffmann Hill, Refugio Pass and Painted Cave, see ref [4], vouchers at UCSB) and material collected by M S Taylor (in Butte Co , CA, vouchers at CHSC and at Darmstadt) This material was more or less identical on TLC and was therefore combined for work-up Fronds of two different additional populations of var *triangularis* were collected by E Wollenweber at School Land Gulch at the Pardee Reservoir, CA in July 1980 (vouchers EW-2 and EW-3 at Darmstadt) Material of var *viscosa* was also collected by D M Smith (vouchers 43352 and 43383 at USCB)

The dried fronds were rinsed with Me_2CO to dissolve the exudate material Most of the ceroptene, the major constitu-

ent of the farina on ceroptene-type plants, was removed by crystallization from EtOH. The remainder was chromatographed on columns of silica gel, then on polyamide Elution was with toluene and increasing quantities of MeCOEt and MeOH The fractions thus obtained were still mixtures They were separated further by repeated CC on smaller columns or by prep TLC on silica gel and on polyamide, respectively Solvents used for polyamide (DC-11) were. (A) petrol (100–140°)–toluene–MeCOEt–MeOH (90 30 2 1 5), (B) toluene–petrol (100–140°)–MeCOEt–MeOH (60 30 10 5), (C) toluene–dioxane–MeOH (8 1 1) For silica we used (D) toluene–MeCOEt (9 1) and (E) toluene–dioxane–HOAc (90.25 4) Evaluation was under UV 366 before and after spraying with Naturstoffreagenz A (C Roth, Karlsruhe, abbrev NA) ^{1}H NMR spectra were recorded in DMSO with TMS as internal standard Mps are uncorr

Spectral data UV spectra were measured in MeOH with the classical reagents [34] λ_{max} is in nm MS data are m/z values (rel int). ^{1}H NMR data are in ppm/TMS, measured in DMSO-d_6 (90 MHz)

5 UV (355), 325, (300), 271, $AlCl_3$ 410, 350, 309, 280; NaOAc 393, 281, NaOAc + H_3BO_3 370, 280 MS 330 $[M]^+$ (64) 329 (9), 316 (17), 315 (100), 302 (11), 287 (8), 272 (12), 196 (4), 181 (39), 167 (7), 154 (17), 121 (31), 107 (55), 105 (16). ^{1}H NMR 12 5 (1H, *s*, OH-5), 10 4 (2H, *br* signal, 2 OH), 7 95 and 6 98 (2H each, *d*, AA'BB' spin system B-ring *p*-substituted), 6 28 (1H, *s*, H-6), 3 79 (3H, *s*, OMe), 3 81 (3H, *s*, OMe).

6 UV 373, 321, 273, $AlCl_3$ 434, 356, (314), 273, NaOH 417, (320), 282, 258, NaOAc 400, (305), 230; NaOAc + H_3BO_3 385, (310), 227 MS 330 $[M]^+$ (63), 329 (27), 316 (17), 315 (100), 301 (6), 287 (6), 165 (10), 139 (11), 135 (16) ^{1}H NMR 12.1 (1H, *s*, OH-5), 8 13 and 7 16 (2H each, *d*, AA'BB'), 6 29 (1H, *s*, H-6), 3 85 (3H, *s*, OMe), 3 82 (3H, *s*, OMe)

7 UV (360), 323, 276, $AlCl_3$ 415, 350, 312, 285, NaOH 385, (305), 285, NaOAc 386, 281, NaOAc + H_3BO_3 368, 280 MS 344 $[M]^+$ (34), 343 (4), 329 (62), 167 (8), 139 (21), 135 (19), 119 (20), 77 (42) ^{1}H NMR 12 34 (1H, *s*, OH-5), 8 04 and 7 18 (2H each, *d*, AA'BB'), 6 99 (1H, *s*, H-6), 3 86 (3H, *s*, OMe), 3 80 (6H, *s*, 2 OMe).

8 UV 362, 326, 274, $AlCl_3$ 419, 356, 275, 251, NaOH 410, (350), 280; NaOAc 405, (330), 283 MS 284 $[M]^+$ (100), 283 (24), 256 (8), 255 (13), 153 (10), 105 (30), 77 (70). ^{1}H NMR 12 6 (1H, *br* signal, OH-5), 8 2 (2H, *m*, H-2', H-6'), 7 53 (3H, *m*, H-3', H-4', H-5'), 6 53 (1H, *s*, H-6), 2 0 (3H, *s*, Me)

9 UV 323, 274, $AlCl_3$ 342, 285, NaOH 368, 275, NaOAc 363, 275, NaOAc + H_3BO_3 326, 274 MS 298 $[M]^+$ (96), 297 (100), 280 (14), 279 (28), 269 (10), 267 (12), 255 (15), 105 (24), 77 (38). ^{1}H NMR 12 7 (1H, *br* signal, OH-5), 8 05 (2H, *m*, H-2', H-6'), 7 62 (3H, *m*, H-3', H-4', H-5'), 6 57 (1H, *s*, H-6), 3 88 (3H, *s*, OMe), 2 09 (3H, *s*, Me)

10 UV 359, 325, 274, $AlCl_3$ 415, 359, 276, 255, 230; NaOH 405, (335), 274, 250, 221, NaOAc 408, (335), 275, NaOAc + H_3BO_3 360, 325, 274, MS 298 $[M]^+$ (100), 297 (43), 280 (8), 269 (25), 255 (7), 105 (25), 77 (28) ^{1}H NMR 12 27 (1H, *s*, OH-5), 9 7 (1H, *br* signal, OH-3), 7 89 (2H, *m*, H-2', H-6'), 7 62 (3H, *m*, H-3', H-4', H-5'), 6 92 (1H, *s*, H-6), 3 93 (3H, *s*, OMe), 2.02 (3H, *s*, Me)

11 UV 386, (327), 285, $AlCl_3$ 435, 361, 285, 245, NaOH 377, 261, NaOAc 385, 283 MS 314 $[M]^+$ (100), 313 (10), 299 (14), 269 (13), 285 (21), 271 (24), 105 (55), 77 (25) ^{1}H NMR 12 0 (1H, *s*, 5-OH), 8 28 (2H, *m*, H-2', H-6'), 7 55 (3H, *m*, H-3', H-4', H-5'), 3 87 (3H, *s*, OMe), 2.11 (3H, *s*, Me)

12 UV 375, 285, $AlCl_3$ 370, 285 MS. 328 $[M]^+$ (100), 327 (58), 309 (13), 285 (28), 270 (14), 195 (15), 115 (33), 105 (45), 77 (60) ^{1}H NMR 12 14 (1H, *s*, OH-5), 9 33 (1H, *s*, OH-8), 8 08 (2H, *m*, H-2', H-6'), 7 55 (3H, *m*, H-3', H-4', H-5'), 3 84 (3H, *s*, OMe), 3 78 (3H, *s*, OMe), 2 06 (3H, *s*, Me)

13 UV 369, (325), 280; $AlCl_3$ 426, 356, 312, 280, NaOH 424, 330, 290; NaOAc 408, 333, 290; NaOAc + H_3BO_3 (375), 330, 287 MS 298 $[M]^+$ (59), 297 (13), 270 (36), 269 (23), 193 (40), 156 (55), 105 (14), 77 (81) ^{1}H NMR 12 51 (1H, *s*, OH-5), 8 2 (2H, *m*, H-2', H-6'), 7 52 (3H, *m*, H-3', H-4', H-5'), 2.23 (3H, *s*, Me), 2 07 (3H, *s*, Me).

15 UV 374, 322, 277, $AlCl_3$ 428, 358, 280, 255, NaOH 413, (336), 253 MS 312 $[M]^+$ (100), 311 (18), 294 (50), 283 (17), 191 (10), 156 (20), 105 (40), 77 (49) ^{1}H NMR. 12 25 (1H, *br* signal, OH-5), 9 8 (1H, *br* signal, OH-7), 8 22 (2H, *m*, H-2', H-6'), 7 55 (3H, *m*, H-3', H-4', H-5'), 3 50 (3H, *s*, OMe), 2 35 (3H, *s*, Me), 2 13 (3H, *s*, Me)

16 UV 355, 279; $AlCl_3$ (410), 363, 313, 289, NaOH 405, 334, 284, NaOAc 390, (340), 281, NaOAc + H_3BO_3 335, 280 MS 328 $[M]^+$ (100), 327 (63), 309 (32), 299 (16), 285 (40), 181 (8), 150 (26), 131 (28), 121 (33) ^{1}H NMR 12 83 (1H, *s*, OH-5), 10 2 (2H, *br* signal, 2 OH), 7 96 and 6 96 (2H each, *d*, AA'BB'), 3 78 (3H, *s*, OMe), 224 (3H, *s*, Me), 2 04 (3H, *s*, Me)

18a UV (338), 295, $AlCl_3$ (345), (318), 294, NaOH (347), 293, NaOAc (328), 293 MS 284 $[M]^+$ (100), 283 (61), 207 (98), 180 (100), 152 (87), 109 (50), 104 (18), 103 (24), 77 (25) ^{1}H NMR 12 3 (1H, *s*, OH-5), 7 45 (5H, *m*, B-ring), 6 26 (1H, *s*, H-8), 5 61 (1H, *dd*, H-2, $J_{H\text{-}2'/H\text{-}3a}$ = 12 Hz, $J_{H\text{-}2/H\text{-}3b}$ = 3 8 Hz), 3 33–2 86 (2H, *m*, H-3a, H-3b, $J_{H\text{-}3a/H\text{-}3b}$ = 17 Hz), 3 84 (3H, *s*, OMe), 2 11 (3H, *s*, Me)

18b UV (340), 291, $AlCl_3$ 387, 315, NaOH (340), 290; NaOAc 340, 292 MS 284 $[M]^+$ (52), 283 (20), 207 (38), 180 (54), 152 (52), 109 (21), 104 (28), 103 (20), 43 (100) ^{1}H NMR 12 16 (1H, *s*, OH-5), 7 46 (5H, *m*, B-ring), 6 20 (1H, *s*, H-6), 5 61 (1H, *dd*, H-2, $J_{H\text{-}2/H\text{-}3a}$ = 12 Hz, $J_{H\text{-}2/H\text{-}3b}$ = 4 Hz), 3 2–2.9 (2H, *m*, H-3a, H-3b, $J_{H\text{-}3a/H\text{-}3b}$ = 17 Hz), 3 82 (3H, *s*, OMe), 1 88 (3H, *s*, Me)

20: UV 347, 280; $AlCl_3$ 346, 279, NaOH 393, 289, NaOAc 386, 285, NaOAc + H_3BO_3 346, 280 MS 316 $[M]^+$ (43), 210 (22), 195 (100), 168 (40), 121 (8), 120 (10), 107 (52) ^{1}H NMR 11 35 (1H, *br* signal, OH-6'), 9 10 (1H, *s*, OH-4), 7 00 and 6 62 (2H each, *d*, AA'BB'), 3 61 (3H, *s*, OMe), 3 30 (2H, tr) and 2 76 (2H, tr) = $-CH_2-CH_2-$ bridge, 1 98 (6H, *s*, 2 Me)

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REFERENCES

1 Blasdale, W C (1893) *Erythea* **1**, 252
2 Blasdale, W C (1903) *J Am Chem Soc* **25**, 1141
3 Nilsson, M (1959) *Acta Chem Scand* **13**, 750
4 Smith, D M, Craig, S P and Santarosa, J (1971) *Am J Botany* **58**, 292
5 Wollenweber, E and Smith, D M (1981) *Am Fern J* **71**, 120
6 Star, A E, Rösler, H, Mabry, T J and Smith, D M (1975) *Phytochemistry* **14**, 2275
7 Star, A E, Mabry, T J and Smith, D M (1978) *Phytochemistry* **17**, 586
8 Dietz, V H, Wollenweber, E, Favre-Bonvin, J and Smith, D M (1981) *Phytochemistry* **20**, 1167
9 Smith, D M (1980) *Bull Torrey Bot Club* **107**, 134
10 Wollenweber, E., Dietz, V H, Smith, D M and Seigler, D S (1979) *Z Naturforsch Teil C* **34**, 876
11 Wagner, H, Maurer, G, Farkas, L, Hänsel, R and Ohlendorf, D (1971) *Chem Ber* **104**, 2381

12 Wollenweber, E (1982) in *The Flavonoids—Advances in Research* (Harborne, J B and Mabry, T J, eds), pp 189–261 Chapman & Hall, London
13 Goudard, M and Chopin, J (1976) *C R Acad Sci Ser C* **282**, 683
14 Tandon, S and Rastogi, R. P (1977) *Phytochemistry* **16**, 1455
15 Horie, T, Kourai, T, Osaka, H and Nakayama, M (1982) *Bull Chem. Soc Jpn* **55**, 2933
16 Wagner, H, Chari, V M and Sonnenbichler, J (1976) *Tetrahedron Letters* **21**, 1799
17 Markham, K R and Ternai, B (1976) *Tetrahedron* **32**, 2607
18 Österdahl, B G (1976) *Acta Chem. Scand Sect B* **32**, 93
19 Markham, K R, Ternai, B, Stanley, R, Geiger, H and Mabry, T J (1978) *Tetrahedron* **34**, 1389
20 Rabesa, Z A, Voirin, B, Favre-Bonvin, J and Lebreton, P (1978) *Phytochemistry* **17**, 1810
21 Wollenweber, E (1982) *Phytochemistry* **21**, 1462
22 Wollenweber, E, Dietz, V H, Schillo, D and Schilling, G (1980) *Phytochemistry* **19**, 2043
23 Wollenweber, E, Walter, J and Schilling, G (1981) *Z Pflanzenphysiol* **104**, 161
24 Wollenweber, E and Dietz, V H (1981) *Phytochemistry* **20**, 1167
25 Rabesa, Z A. (1980) Doctoral Thesis, Université Claude Bernard, Lyon-I
26 Ghisalberti, E L, Jefferies, P R and Stacey, C I (1967) *Aust J Chem.* **20**, 1049
27 Nagarajan, G R and Seshadri, T R (1964) *Phytochemistry* **3**, 477
28 Krishnamurty, M, Seshadri, T R and Shankaran, P R (1965) *Tetrahedron* **22**, 941
29 Dawson, R M, Henrick, C A, Jefferies, P R and Middleton, E J (1965) *Aust J Chem.* **18**, 1871
30 Hitz, C, Mann, K and Wollenweber, E (1982) *Z Naturforsch Teil C* **37**, 337
31 Dietz, V H (1980) Doctoral Thesis, TH Darmstadt
32 Wollenweber, E and Dietz, V H (1980) *Biochem Syst Ecol* **8**, 21
33 Wollenweber, E and Dietz, V H (1980) *Biochem. Syst Ecol* **8**, 457
34 Mabry, T J, Markham, K R and Thomas, M B (1970) *The Systematic Identification of Flavonoids* Springer, Berlin