Phytochemistry, 1981, Vol. 20, pp. 527–529. Pergamon Press Ltd. Printed in England. 0031–9422/81/030527–03 $02.00/0

FLAVANONE AND OTHER CONSTITUENTS FROM *ONYCHIUM SILICULOSUM**

TIAN-SHUNG WU, CHENG-SHENG KUOH, SHIEN-TSONG HO, MEEI-SHIEU YANG and KONG-KO LEE

Chia-Nan Junior College of Pharmacy, Tainan 717, Taiwan, Republic of China

(*Received* 30 *May* 1980)

Key Word Index—*Onychium siliculosum*; Pteridaceae; pterosins; flavanones; structure elucidation.

Abstract—Two new compounds, onitinoside and onysilin along with the known compounds, pinostrobin, onitin, onitisin, campesterol, sitosterol and *n*-alkanes (C_{25}–C_{33}) were isolated from *Onychium siliculosum*. Onitinoside and onysilin were identified by spectral and chemical methods as 4-*O*-glucosyl-6-(2′-hydroxyethyl)-2,2,5,7-tetramethyl-indan-1-one and 5-hydroxy-6,7-dimethoxyflavanone, respectively.

INTRODUCTION

In the folk medicine of Formosa, *Onychium* has been used for treating enteritis, dysentery, abdominal cramps and pyrexia [1, 2]. We were interested in the isolation of bioactive compounds because the crude EtOH extract caused blockage of peristaltic reflex of the guinea-pig ileum (Ho, Yang, Wu and Lee, unpublished results). We now report the isolation and structure elucidation of two new compounds, onitinoside and onysilin, along with five known compounds from the whole herb of *O. siliculosum* (Desv.) C. Chr.

RESULTS AND DISCUSSION

The ethanolic extract of the powdered whole herb of *O. siliculosum* was successively partitioned with $CHCl_3$, EtOAc and *n*-BuOH. Subsequent separations of the $CHCl_3$ and EtOAc extracts were achieved using Si gel column chromatography affording eleven compounds **1**–**11**, respectively.

Compound **5a** had pale yellow needles, mp 150–2° (Me_2CO), $C_{17}H_{16}O_5$ (M^+, 300). It gave a greyish green colour with $FeCl_3$ and an orange colour with Mg–HCl, characteristic of a flavonoid. The UV spectrum showed maxima at 211,230(sh), 288 and 338. Both the occurrence of a 30 nm bathochromic shift on the addition of $AlCl_3$ and bands at 3270 and 1655 cm^{-1} in the IR confirmed the occurrence of a hydroxyl chelated to carbonyl. The flavanone nucleus was confirmed by the presence in the NMR spectrum of an ABX system centred at δ 2.88, 3.02 and 5.40 for the C-2 and C-3 protons. Signals at δ 3.84 (3H) and 3.88 (3H) could be assigned to two OMe substituents and a hydrogen bonded OH resonating at δ 11.95. The remaining six protons were observed as a 5H singlet at δ 7.44 and a 1H signal at δ 6.12. This spectrum is compatible with that anticipated for dimethoxy-monohydroxy flavanone with all the substituents on the A-ring. The absence of B-ring substitution was confirmed by the presence of ions at *m/e* 196 ($C_9H_8O_5$) and *m/e* 104 (C_8H_8) in the MS [6]. From the above data, we could assign the structure of **5a** as 5-hydroxy-6,7-dimethoxyflavanone or 5-hydroxy-7,8-dimethoxyflavanone. These two compounds have been previously synthesized [4]. Owing to lack of authentic reference material, **5a** was further methylated to the corresponding trimethoxyflavanone whose mp and UV data were identical with 5,6,7-trimethoxyflavanone which had been previously reported by Krishnamurty *et al.* [4] and were different from the spectra of the 5,7,8-trimethoxy analogue [7]. Therefore, the structure of **5a** was assigned as 5-hydroxy-6,7-dimethoxyflavanone. We propose the name onysilin for this compound.

Compound **8a** had colourless crystals, mp 172–4° (EtOAc). $C_{21}H_{30}O_8$ (M^+ 410). Fehling's test was positive. The UV spectrum showed maxima at 222, 264, 308 nm with no shift upon addition of NaOH solution. These characteristics indicate that **8a** is a 1-indanone glycoside lacking a free phenolic hydroxyl [6]. **8a** was hydrolysed with conc HCl to the corresponding aglycone (onitin **6a**, mp 220–1°) and sugar (glucose). Acetylation of **8a** afforded a colourless syrupy liquid (**8b**). The NMR spectrum of **8b** showed five acetyl group signals at δ 1.98, 2.00, 2.08. From the above data we could assign the structure of **8a** as onitin-4-glucoside. It is a new compound which we name onitinoside.

The other compounds were identified by comparison with authentic samples of: pinostrobin [3], onitin [5], onitisin [5], campesterol, sitosterol and *n*-alkanes (C_{25}–C_{33}) by mp, IR, TLC, NMR, GC and UV. Studies of compounds **2**, **9**, **10** and **11** are still in progress. Onitin and onitinoside showed pharmacological activity in blocking the peristaltic reflex of the guinea-pig ileum, in inhibition of the responses of guinea-pig ileum to histamine and of inhibition of the responses of guinea-pig tracheal muscle to histamine.

* Part XI in the series 'Constituents of Formosan Folk Medicine'. For Part X see Wu, T.-S., Chen, M.-T., Kuoh, C.-S. and Yang, J.-J. (1980) *J. Chin. Chem. Soc.* (in press).

EXPERIMENTAL

Mps were uncorr., TMS was used as internal standard for all NMR spectra which were recorded in $CDCl_3$ unless otherwise stated. MS spectra were taken with a direct inlet system. The GC conditions were described in a previous paper [8, 9].

Plant material. O. siliculosum was collected from Liu-Kuei, Kaoshiung, Taiwan and verified by Professor C.-S. Kuoh and the specimen is deposited in the Herbarium of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, Republic of China.

Extraction and separation. The EtOH extract of the powdered whole herbs of *O. siliculosum* (2.5 Kg) were treated with $CHCl_3$ and H_2O. The $CHCl_3$ extract was evapd to dryness and subjected to Si gel column chromatography by eluting successively with *n*-C_6H_{12}, C_6H_6 and C_6H_6–Me_2CO to afford compounds **1**–**6**. The aq. layer was extracted with EtOAc and *n*-BuOH. The EtOAc extract was separated on Si gel column and eluted with $CHCl_3$, $CHCl_3$–MeOH, MeOH to obtain compound **7**–**11**.

n-Alkanes (C_{25}–C_{33}) (**1**). Colourless crystals (Me_2CO), mp 65–7°, γ_{max}^{KBr} cm^{-1}: 2920, 2850, 1460, 730, 720; Br_2 and $KMnO_4$ test (−), composition: *n*-$C_{25}H_{52}$ (1%), *n*-$C_{26}H_{54}$ (2%), *n*-$C_{27}H_{56}$ (15%), *n*-$C_{28}H_{58}$ (5%), *n*-$C_{29}H_{60}$ (30%), *n*-$C_{30}H_{62}$ (9%), *n*-$C_{31}H_{64}$ (27%), *n*-$C_{32}H_{66}$ (6%), *n*-$C_{33}H_{68}$ (5%), yield 1.245 g.

Campesterol and sitosterol(**3**). Colourless crystals, mp 136–8° (Me_2CO), Liebermann Burchard test (+), γ_{max}^{KBr} cm^{-1}: 3300, 1040 (OH), 1460, 1380, 1360; composition: campesterol (11.2%), sitosterol (89.8%), yield 2.125 g.

Pinostrobin (**4**). Colourless crystals mp 102–4° (Me_2CO), Mg–HCl and $FeCl_3$ test were positive, Calc. for $C_{16}H_{14}O_4$, C: 71.10, H: 5.22, Found: C: 70.79, H: 5.12; MS: 270 (M^+, 100). It was identified by comparison of the NMR, IR, and UV spectra with the standard spectra of Suga [4], yield 2.304 g.

Onysilin (*5-hydroxy-6,7-dimethoxyflavanone*, **5a**). Pale yellow needles, mp 150–2° (Me_2CO), Mg–HCl and $FeCl_3$ test were positive; Calc. for $C_{17}H_{16}O_5$, C: 67.99, H: 5.37, Found: C: 67.94, H: 5.36; λ_{max}^{MeOH} nm (log ε): 211 (4.28), 230 (sh, 4.06), 288 (4.09), 338 (3.32). $\lambda_{max}^{+AlCl_3}$ nm (log ε): 223 (sh, 4.29), 314 (4.22), 368 (3.52), $\lambda_{max}^{+AlCl_3+HCl}$ nm (log ε): 223 (sh, 4.28), 312 (4.25), 366 (3.56), λ_{max}^{+NaOMe} nm (log ε): 240 (4.06), 290 (4.08), 367 (3.60), λ_{max}^{+NaOAc} nm (log ε): 288 (4.12), 338 (3.45); γ_{max}^{KBr} cm^{-1}: 3270 (OH), 1655 (C = O), 1634, 1585 (C = C); NMR δ: 2.88 (1H, *d*, *J* = 4 Hz, C_3-*cis*), 3.02 (1H, *d*, *J* = 12 Hz, C_3-*trans*), 3.84 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 5.40 (1H, *q*, *J* = 4 and 12 Hz, C_2), 6.12 (1H, *s*, C_8), 7.44 (5H, *s*, B-ring Ar), 11.95 (1H, *s*, OH). MS: 300 (M^+, 97), 299, 285, 233, 196 (70), 181 (100), 104, yield 0.215 g.

O-Methylonysilin (**5b**). 100 mg **5a** was methylated with CH_2N_2 to afford 95 mg **5b** as colourless crystals mp 159–60° (Me_2CO). Calc. for $C_{18}H_{18}O_5$, C: 68.78, H: 5.77, Found: C 68.73, H: 5.74; λ_{max}^{MeOH} nm (log ε): 228 (4.31), 277 (4.16), 323 (3.66); γ_{max}^{KBr} cm^{-1}: 1685 (C = O), 1617, 1575 (C = C); NMR (δ): 2.86 (1H, *d*, *J* = 4 Hz, C_3-*cis*), 2.96 (1H, *d*, *J* = 12 Hz, C_3-*trans*), 3.85 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 3.97 (3H, *s*, OMe), 5.42 (1H, *q*, *J* = 4 and 12 Hz, C_2), 6.36 (1H, *s*, C_8), 7.46 (5H, *s*, B-ring Ar). MS; 314 (M^+, 63%), 313, 299, 237, 210 (100%), 195 (61%), 104.

Onitin **6a**. Colourless crystals, mp 220–2° (MeOH), (lit. 214°) [5], Calc. for $C_{15}H_{20}O_3$, C: 72.55, H: 8.12, Found: C: 72.34, H: 8.26. NMR δ (C_5D_5N): 1.15 (6H, *s*, 2 × Me), 2.55 (3H, *s*, Me), 2.81 (3H, *s*, Me), 2.95 (2H, *s*, C_3), 3.17 (2H, *t*, C-1′), 3.95 (2H, *t*, C-2′), 10.37 (1H, *s*, OH), 5.56 (1H, br., OH); δ (DMSO-d_6): 1.10 (6H, *s*, 2 × Me), 2.23 (3H, *s*, Me), 2.50 (3H, *s*, Me), 2.74 (2H, *s*, C-3), 2.80 (2H, *t*, C-1′), 3.30 (2H, *t*, C-2′), 3.55 (1H, br., OH), 8.54 (1H, *s*, OH), MS: 248 (M^+, 62%), 217 (M-31, 81%). It was identical by comparison of the mp, IR and UV, with an authentic sample, yield 3.762 g.

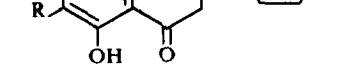
O-Methylonitin (**7b**). Colourless crystals, mp 115–7° (Me_2CO), Calc. for $C_{16}H_{22}O_3$, C: 73.25, H: 8.71; Found: C: 72.47, H: 8.71; λ_{max}^{MeOH} nm: 258, 303; γ_{max}^{KBr} cm^{-1}: 3500 (OH), 1710 (C = O), 1600 (C = C); NMR (δ): 1.20 (6H, *s*, 2 × Me), 2.10 (1H, *s*, OH), 2.36 (3H, *s*, Me), 2.63 (3H, *s*, Me), 2.90 (2H, *s*, C-3), 3.02 (2H, *t*, C-1′), 3.75 (2H, *t*, C-2′), 3.77 (3H, *s*, OMe). MS: 262 (M^+), 247 (100%, $M^+ - 15$), 231 ($M^+ - 31$).

Onitisin (**8**). Colourless crystals, mp 185–7° (EtOAc) (lit. 184° [5], Calc. for $C_{15}H_{20}O_4$; C, 68.16, H: 7.63, Found C: 67.98, H: 7.82. MS: 264 (M^+, 10%), 249, 234, 233, 231, 31 (100%). It was identified by comparison of the mp, IR and TLC with authentic sample, yield 0.078 g.

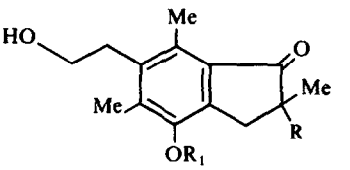
Onitinoside (**8a**). Colourless crystals, mp 172–4° (EtOAc), Calc. for $C_{21}H_{30}O_8.H_2O$, C: 58.86, H: 7.53, Found: C: 58.56, H: 7.42; λ_{max}^{MeOH} nm (log ε): 222, (4.29), 264, (4.08), 308 (3.41); λ_{max}^{NaOH} nm: 217, 262, 308. ν_{max}^{KBr} cm^{-1}: 3270–3420 (OH), 1675 (C = O), 1590 (C = C). NMR δ (C_5D_5N): 1.16 (3H, *s*, Me), 1.24 (3H, *s*, Me), 2.79 (6H, *s*, 2 × Me), 3.14 (2H, *t*, *J* = 8 Hz, C-1′), 3.27 (2H, *s*, C-3), 3.94 (2H, *t*, *J* = 8 Hz, C-2′), 5.29 (1H, *d*, *J* = 6 Hz, C-2′), 5.29 (1H, *d*, *J* = 6 Hz, anomeric-H), 3.46–4.41 (11H, glucosyl-H), 6.01 (1H, *br.*, OH). MS: 410 (M^+), 248, 247 (85%), 233 (100%), 217 (51%), 31 (91%), yield 6.107 g.

Onitinoside pentaacetate (**8b**). Treatment of 200 mg of **8a** with 1 ml of Ac_2O and pyridine, afforded **8b** as a colourless syrupy liquid. NMR δ: 1.11 (3H, *s*, Me), 1.15 (3H, *s*, Me), 1.98, 2.00, 2.08 (15 H, 5 × Ac), 2.31 (3H, *s*, Me), 2.60 (3H, *s*, Me), 2.86 (2 H, *s*, C-3), 2.98 (2 H, *t*, *J* = 8 Hz, C-1), 4.06 (2 H, *t*, *J* = 8 Hz, C-2′), 3.80–5.35 (glucosyl-H), MS: 620 (M^+), 577, 331, 290, 289, 271,247,230,169,109, 33 (100%), 31.

Hydrolysis of onitinoside (**8a**). 0.2 g of **8a** was refluxed with C-HCl for 10 mins, the ppt was filtered and recrystallized with MeOH to yield colourless crystals, mp 220–1°. It was identified as onitin (**6a**) by comparison if the IR, mp and TLC with authentic onitin. The sugar residue was subjected to PPC in the usual manner and identified as glucose.



4 R = H
5a R = OMe



6a R = Me, R_1 = H
7 R = CH_2OH, R_1 = H
8a R = Me, R_1 = Glc.

Acknowledgements—The authors thank Professor T. Nonaka (Tokyo Institute of Technology), Professor N. Morita (Toyama Medical and Pharmaceutical University) for the measurement of NMR, MS, and elemental analysis, and Drs Y. Hayashi, A. Banerji, K. Panichpol and T. Suga for authentic samples and IR, NMR, UV spectra of pinostrobin and 5,7,8-trimethoxyflavanone.

REFERENCES

1. Li, H. H. and Liu, K. C. (1973) *A Survey of the Medical Plants in Taiwan*. p. 6. National Research Institute of Chinese Medicine, Taiwan.
2. Kan, W. S. (1968) *Manual of Vegetable Drugs in Taiwan*. Part III, p. 4. The Chinese Medicine Publishing Inc., Taiwan.
3. Suga, T., Iwata, N. and Asakawa, Y. (1972) *Bull. Chem. Soc. Jpn.* **45**, 2058.
4. Krishanamurty, H. G. and Seshadri, T. R. (1959) *J. Sci. Ind. Res.* **18B**, 151.
5. Banerji, A., Ramakrishnan, G. and Chadha, M. S. (1974) *Tetrahedron Letters* 1369.
6. Mabry, T. J. and Markham, K. R. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds.) Chap. 3. Chapman and Hall, London.
7. Panichpol, K. and Waterman, P. G. (1978) *Phytochemistry* **17**, 1363.
8. Wu, T. S., Chen, M. T., Kuoh, C. S. and Yang, J. J. (1980) *J. Chin. Chem. Soc.* (in press).
9. Wu, T. S., Yeh, M. Y. and Chen, B. Y. (1980) *J. Taiwan Pharm. Assoc.* (in press).