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New coumarins from *Harbouria trachypleura*: isolation and synthesis

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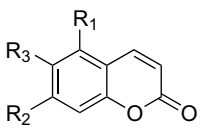
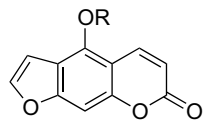
Abstract—Two known furanocoumarins, (+)-oxypeucedanin and (+)-saxalin, two known coumarins, umbelliferone and (+)-epoxysuberosin, and two new coumarins (+)-trachypleuranin-A and (±)-trachypleuranin-B, were isolated from the methanol extract of *Harbouria trachypleura*. An efficient five-step asymmetric synthesis of (+)-trachypleuranin-A was performed using a sequential [3,3] Claisen–Cope rearrangement and a Shi asymmetric epoxidation. (±)-Trachypleuranin-B, a chlorinated coumarin, was synthesized from (±)-epoxysuberosin.

Our research laboratories are currently investigating bacterial MDR (multidrug resistance) efflux pump inhibitors of the gram-positive bacterium *Staphylococcus aureus*.[1] We recently extended the scope of this project to include the gram-negative bacteria *Pseudomonus aeruginosa* and *Escherichia coli*. A preliminary investigation with *E. coli* mutants showed that coumarins may potentiate the action of anthraquinones as MDR efflux pump inhibitors.[2] *Harbouria trachypleura* (A. Gray) J. Coulter & Rose belongs to the Apiaceae (Umbelliferae), a family of plants known to often contain coumarins. *H. trachypleura*, commonly called





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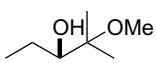
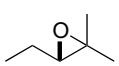
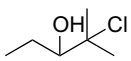
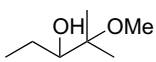
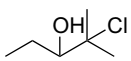
2: $R_1 = H$, $R_2 = OMe$, $R_3 =$ 

3: $R_1 = H$, $R_2 = OMe$, $R_3 =$ 

4: $R_1 = H$, $R_2 = OMe$. $R_3 =$ 

7: $R_1 = R_2 = OMe$, $R_3 =$ 

8: $R_1 = R_2 = OMe$, $R_3 =$ 

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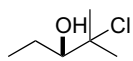
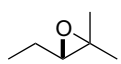
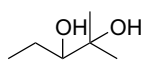
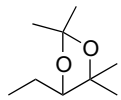
5: R = 

6: R = 

14: R = 

14a: R= 

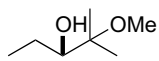
15: R = 

Figure 1. Coumarins and furanocoumarins from *Harbouria trachypleura* (**1**–**6**) and other representative structures.

Keywords: Apiaceae; *Harbouria trachypleura*; coumarins; furanocoumarins; Shi asymmetric epoxidation; [3,3] Claisen–Cope rearrangement.
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whiskbroom parsley, is approximately 30–50 cm tall and grows on the foothills of the Colorado and Wyoming Front Range.[3] Our assays showed that *H. trachypleura* does not contain bacterial MDR efflux pump inhibitors of *E. coli*; however, the plant belongs to a monotypic genus and this distinction warranted its further investigation. In this letter, we report the isolation of four coumarins (**1**–**4**) and two furanocoumarins (**5**, **6**) (Fig. 1) from the methanol extract of *H. trachypleura* and the synthesis of **2**, **3,** and **4**.

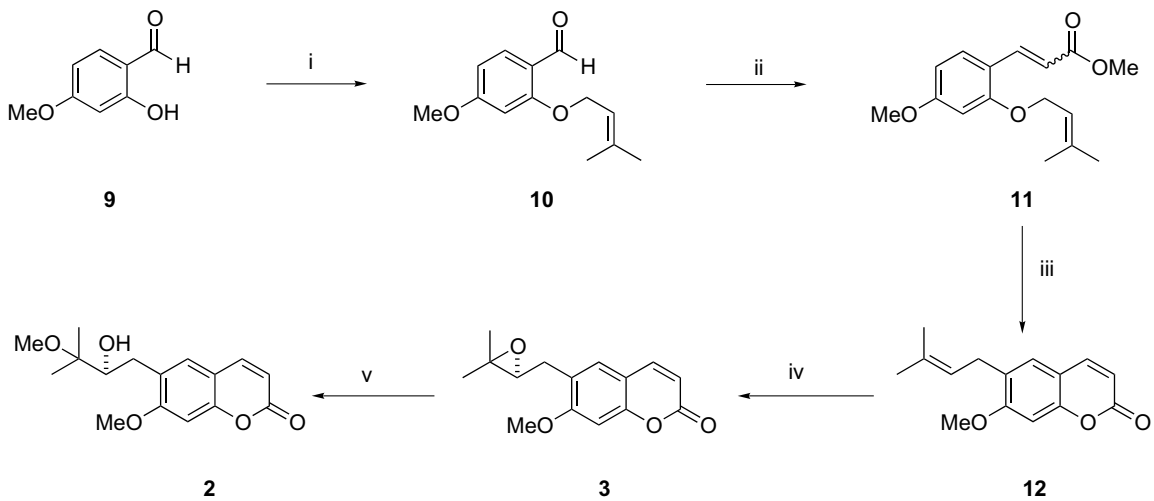
Isolation

The dried aerial parts of *H. trachypleura* (185.6 g) were immersed in 1 L of distilled MeOH for one day, the MeOH filtered, and the process repeated another time. A portion (9.61 g) of the total crude MeOH extract (18.10 g) was subjected to VLC (vacuum liquid chromatography, gradient hexanes to EtOAc) to yield 14 fractions. Fractions 5–7 contained coumarins as determined by TLC and ^{1}H NMR. Fraction 7 was subjected to flash column chromatography (CC, 97:3 CH_2Cl_2/$(CH_3)_2CO$) to yield 10 mg of umbelliferone (**1**). Combined fractions 5 and 6 were also subjected to CC (97:3 to 4:1 CH_2Cl_2/$(CH_3)_2CO$) to yield two fractions A (frs. 5–10) and B (frs. 11–16). Both frs. A and B were chromatographed separately on C-18 VLC (1:1 MeOH/H_2O to 100% MeOH, in-house prepared C-18) to provide twelve fractions each. Fr. A1 yielded 4 mg of a new coumarin, **2**. Fr. A2 yielded 7 mg of epoxysuberosin (**3**). Fr. A3 yielded a mixture of another new coumarin (**4**) and saxalin (**5**). These isolates were separated by preparative TLC using 3:2 hexanes/EtOAc to yield 3 mg of **4** and 2 mg of **5**. Fr. B1 yielded 75 mg of oxypeucedanin (**6**). Final isolates (**2**, **3**, and **6**) were subjected to preparative TLC (97:3 CH_2Cl_2/$(CH_3)_2CO$) to remove minor impurities.

Isolates were analyzed by ^{1}H, ^{13}C, COSY and DEPT NMR as well as ES^- and FAB^+ mass spectrometry. Spectral data for 7-methoxycoumarins with derivatized prenyl side chains are well documented in the literature. The structures for the unknown coumarins were tentatively assigned as **2** and **4** based upon data for similar compounds **7** and **8**.[4,5] Compounds **1**, **3**,[6,7] **4**[8,9] and **6**[10,11] are characterized in the literature and all spectra were in excellent agreement with previously reported isolations.

Synthesis and structure verification of (+)-2, (+)-3, and (±)-4

The synthesis of **2** and **3** is outlined in Scheme 1. The commercially available 2-hydroxy-4-methoxybenzaldehyde (**9**) was used as a starting point. A Williamson ether reaction with 4-bromo-2-methyl-2-butene afforded the phenyl prenyl ether **10** in 80% yield. Still's reagent[12] was used under thermodynamic conditions to smoothly yield the corresponding ester **11** in 96% yield and 77:23 *E*/*Z* selectivity. The optimization of *E*/*Z* selectivity was not investigated because of the subsequent cyclization reaction of **11.** This cyclization was achieved by refluxing **11** in $PhN(Et)_2$ to yield the corresponding 6-prenylated coumarin suberosin (**12**) in good yield. This reaction utilizes the well-known sequential [3,3] Claisen–Cope rearrangement of phenyl prenyl ethers.[13,14] The coumarin **12** was targeted using a similar strategy by Mali and co-workers under less efficient conditions.[15] Cairns, Harwood and Astles also synthesized **12** in their investigations of 6-substituted prenyl, geranyl, and farnesyl analogues of **1**.[16] They, however, used the coumarin herniarin (**13**) as a starting point. This sequential [3,3] Claisen–Cope rearrangement has also been utilized in the syntheses of other natural products as well.[16–19]



Scheme 1. Synthesis of (+)-trachypleuranin-A: (i) 4-bromo-2-methyl-2-butene, K_2CO_3, acetone, reflux, 1.5 h, 80%; (ii) $(CF_3CH_2)_2P(O)CH_2CO_2Me$, NaH, THF, −30°C, 45 min, 98%, 77:23 *E*/*Z*; (iii) *N*,*N*-diethylaniline, reflux, 4 h, 81%; (iv) Shi asymmetric epoxidation, 82% ee, 85% yield; (v) TsOH, MeOH, 15 min, 99%.

With **12** in hand, the olefin was subjected to Shi asymmetric epoxidation conditions[20] to yield the desired epoxide (+)-**3** in 82% yield. The ^{1}H and ^{13}C spectral data of the synthetic epoxide were essentially identical to those of an authentic sample from *H. trachypleura*. The optical rotation of the synthetic was +17 ($c=0.01$, $CHCl_3$). Gray reported an optical rotation of +34 ($c=0.07$, $CHCl_3$) for a sample of **3** isolated from *Coleonema album*[7] and the isolate from *H. trachypleura* had an optical rotation of +27 ($c=0.006$, $CHCl_3$). While the optical rotation of the synthetic epoxide was only in fair agreement with that of the isolate, the determination of the isolate's absolute configuration was the main goal of utilizing the asymmetric epoxidation. Extensive attempts to determine the % ee of the epoxidation reaction using chiral HPLC, GC and europium-based chiral NMR shift reagents were met with failure. It was thus decided that the % ee of the epoxidation would be determined from the final product using the same chiral analytical techniques.

The final product was easily synthesized using catalytic tosic acid in distilled methanol to give (+)-**2** in 99% yield. The epoxide opening should occur without racemization of the secondary alcohol because of the tertiary carbocation formed as a reactive intermediate. The data for synthetic **2** were in excellent agreement with those of the isolate from *H. trachypleura*. The optical rotation of the isolate was +34 ($c=0.003$, $CHCl_3$) and the synthetic +28 ($c=0.01$, $CHCl_3$) thus confirming the absolute structure of the isolate as (*R*)-(+)-**2**.[21]

The final % ee of **2** was also unobtainable after extensive attempts at chiral HPLC and GC and europium-based chiral NMR shift reagents. A Mosher ester of **2** was thus synthesized using **2**, (+)-MTPA, oxalyl chloride and catalytic DMF in dry CH_2Cl_2. The product was analyzed by C-18 HPLC (HP 1090a, Adsorbosphere C-18 5 micro, 250×4.5 mm column, 1:1 acetonitrile/water, 330 nm) and an 82% ee was determined for **2**.

The synthesis of (±)-**4** (Scheme 2) was elaborated through a racemic epoxidation of **12** with *m*CBPA in 89% yield. The chlorohydrin (±)-**4** was prepared from **12** using concentrated HCl in $CHCl_3$ for 30 minutes (72% yield). The ^{1}H and ^{13}C NMR spectral data of synthetic (±)-**4** were identical to those of the natural product.[22]

It is interesting to note that of the pro-chiral isolates (**2**, **3**, **4**, **5**, and **6**), all but one (**4**) possessed optical activity. Furthermore, **4** is a rare example of a chlorinated natural product from a higher plant. Because of the small quantity of isolated **4**, it was possible that the sample concentration of **4** was not high enough to obtain an optical rotation. To verify the presence or lack of optical rotation, a circular dichroism experiment was performed. The spectrum obtained from this experiment showed no circular dichroism scanning from 254 to 450 nm. It was thus concluded that **4** was a racemic isolate.

There has been some debate in the literature on whether epoxide-opened coumarins are actual plant constituents or artifacts of isolation. Large quantities of toddalolactone (**7**), an analogue of **2**, were found in a Soxhlet extraction of *Toddalia asiatica* with refluxing methanol.[23] It was believed that a thermal initiated opening of 5-OMe epoxysuberosin and subsequent trapping of the opened oxirane intermediate yielded **7**. The authors showed that in a reextraction of *T. asiatica* with supercritical CO_2, **7** was in fact a component of the plant, although smaller quantities of **7** were isolated with this extraction method. This problem was not anticipated with *H. trachypleura* because the extraction was performed at room temperature. Another literature report details the isolation of **14a**, the acetonide of **14**, from the acetone extracts of *Eremocitrus glauca*.[24] It was shown experimentally that the naturally occurring citric acid in *E. glauca* catalyzed the formation of **14a**.[24] During the extraction of *H. trachypleura*, a similar acid catalyzed reaction could have opened the epoxide of **3** to yield **2**. It was assumed that if this reaction occurred with **3** it would have also happened with the major furanocoumarin constituent of the plant (**6**) and thus **15** be easily found in the plant extract. The furanocoumarin **15**, however, was not isolated or seen in any ^{1}H NMR spectra. It was also possible that **2** was formed by a silica-gel catalyzed reaction with methanol, but stirring a sample of **3** with methanol and silica gel for 24 h at room temperature gave only starting material by TLC and ^{1}H NMR. We concluded that **2** and by the same reasoning **4** are in fact constituents of *H. trachypleura* and not artifacts of isolation.

Acknowledgements

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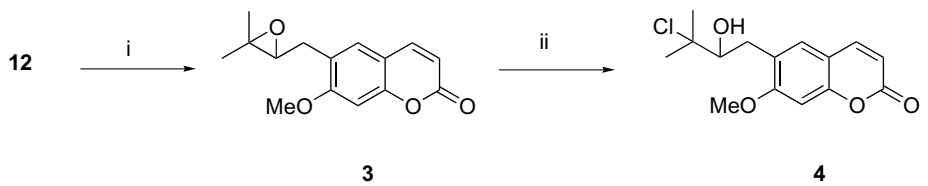

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Scheme 2. Synthesis of (±)-trachypleuranin-B; (i) *m*CPBA, CH_2Cl_2, 30 min, rt, 89%; (ii) conc. HCl, $CHCl_3$, 30 min, rt, 72%.

References

1. (a) Guz, N. R.; Stermitz, F. R.; Johnson, J. B.; Beeson, T. D.; Willen, S.; Hsiang, J.-F.; Lewis, K. *J. Med. Chem.* **2001**, *44*, 261; (b) Guz, N. R.; Stermitz, F. R. *J. Nat. Prod.* **2000**, *63*, 1140; (c) Stermitz, F. R.; Tawara-Matsuda, J.; Lorenz, P.; Mueller, P.; Zenewicz, L.; Lewis, K. *J. Nat. Prod.* **2000**, *63*, 1146; (d) Stermitz, F. R.; Tawara-Matsuda, J.; Lorenz, P.; Zenewicz, L.; Lewis, K. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1433; (e) Hsieh, P.-C.; Siegel, S. A.; Rogers, B.; Davis, D.; Lewis, K. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6602.
2. Unpublished results.
3. Collection occurred near Horsetooth Mountain Park, west of Fort Collins, CO, USA on 5 May 1998. A voucher specimen (#77735) is deposited in the Department of Biology, Colorado State University and identified by Jun Wen, Department of Biology.
4. Reisch, J.; Strobel, H. *Pharmazie* **1982**, *37*, 862–863.
5. Ishii, H.; Kobayashi, J.; Ishikawa, M.; Haginiwa, J.; Ishikawa, T. *Yakugaku Zasshi* **1991**, *111*, 365–375.
6. Nayar, M. N. S.; Bhan, M. K. *Phytochemistry* **1972**, *11*, 3331.
7. Gray, A. I. *Phytochemistry* **1981**, *20*, 1711–1713.
8. Avramenko, L. G.; Nikonov, G. K. *Khim. Prir. Soedin.* **1971**, *7*, 830.
9. Harkar, S.; Razdan, T. K.; Waight, E. S. *Phytochemistry* **1984**, *23*, 419.
10. Lemmich, J.; Pedersen, P. A.; Nielsen, B. E. *Phytochemistry* **1971**, *10*, 3333.
11. Perel'son, M. E.; Sheinker, Y. N.; Syrova, G. P. *Khim. Prir. Soedin.* **1971**, *5*, 576.
12. Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *23*, 4405.
13. Claisen, L.; Tietze, E. *Justus Leibigs Ann. Chem.* **1926**, *449*, 81.
14. Zeigler, F. E. In *Comprehensive Organic Synthesis*; Trost, B. M.; Flemming, I.; Paquette, L. A., Eds.; Pergamon Press: Oxford, 1991; Vol. 5, pp. 875–898.
15. Mali, R. S.; Sandu, P. K.; Manekar-Tilve, A. *J. Chem. Soc., Chem. Commm.* **1994**, 251.
16. Cairns, N.; Harwood, L. M.; Astles, D. P. *J. Chem. Soc., Chem Comm.* **1986**, 1264.
17. Harwood, L. M.; Loftus, G. C.; Oxford, A.; Thomson, C. *Synth. Commun.* **1990**, *20*, 649.
18. Noungoue-Tchamo, D.; Brown, D.; Mariotte, A. *Nat. Prod. Lett.* **1995**, *7*, 73.
19. Gester, S.; Metz, P.; Zierau, O.; Vollmer, G. *Tetrahedron* **2001**, *57*, 1015.
20. Wang, Z.-X.; Tu, Y.; Frohn, M.; Zhang, J.-R.; Shi, Y. *J. Am. Chem. Soc.* **1997**, *119*, 11224. Optimum results were with method B at 25°C.
21. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (*s*, *gem*-Me), 1.25 (*s*, *gem*-Me), 2.48 (*bs*, OH), 2.51 (*dd*, $J=14.0$, 10.0 Hz), 2.99 (*dd*, $J=14.0$, 1.6 Hz), 3.28 (*s*, OMe), 3.72 (*br d*, $J=10$ Hz), 3.91 (*s*, ArOMe), 6.25 (*d*, $J=9.4$ Hz), 6.81 (*s*, ArH), 7.37 (*s*, ArH), 7.64 (*d*, $J=9.4$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.2, 20.7, 31.9, 49.2, 55.9, 76.0, 98.7, 112.0, 113.0, 125.5, 129.5, 143.6, 154.8, 160.7, 161.5. Mp = 113–114°C (uncorrected). HRFAB$^+$ calcd 293.1389, found 293.1399. $[\alpha]_D = +34$ ($c=0.006$, $CHCl_3$), synthetic $[\alpha]_D = +28$ ($c=0.01$, $CHCl_3$).
22. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (*s*, *gem*-Me), 1.71 (*s*, *gem*-Me), 2.25 (*d*, $J=5.2$ Hz, OH), 2.62 (*dd*, $J=14$, 10 Hz), 3.16 (*dd*, $J=10$, 2 Hz), 3.77 (*m*), 3.92 (*s*, OMe), 6.27 (*d*, $J=9.6$ Hz), 6.83 (*s*), 7.34 (*s*), 7.64 (*dd*, $J=9.6$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.6, 29.0, 32.6, 56.0, 75.2, 78.2, 98.9, 112.2, 113.3, 124.5, 129.7, 143.4, 154.9, 160.6, 161.3. Mp = 121–122°C (uncorrected) HRFAB$^+$ calcd 297.0894, found 297.0884. $[\alpha]_D = +0$ ($c=0.003$, $CHCl_3$).
23. Ishii, H.; Tan, S.; Wang, J. P.; Chen, I. S.; Ishikawa, T. *Yakugaku Zasshi* **1991**, *111*, 376–385.
24. Dreyer, D. L.; Huey, P. F. *Phytochemistry* **1974**, *13*, 1237.