SYNTHESIS OF (±)-PRAERUPTORIN A[1] AND RELATED KHELLACTONE DERIVATIVES

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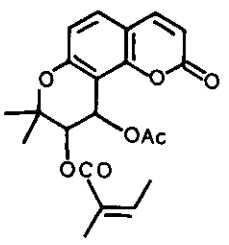
Abstract - The first synthesis of the pyranocoumarin natural product, (±)-praeruptorin A (= Pd-Ia), is described. A general method for the preparation of various khellactone derivatives is reported.

(±)-Praeruptorin A (= Pd-Ia) (1) was isolated by Shibata and Okuyama[2] from the roots of Peucedanum praeruptorum Dunn. (Umbelliferae), which is a traditional Chinese medicine : "Bai-Hua Qian-Hu". The compound 1 was reported to have calcium antagonistic activity[3]. As part of our programme on cardiovascular drugs from natural products, we undertook the synthesis of the title compound to study its profile and to evaluate its potential as a lead on which to base a synthetic programme.



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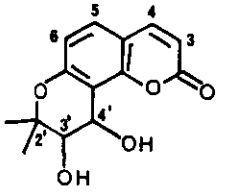

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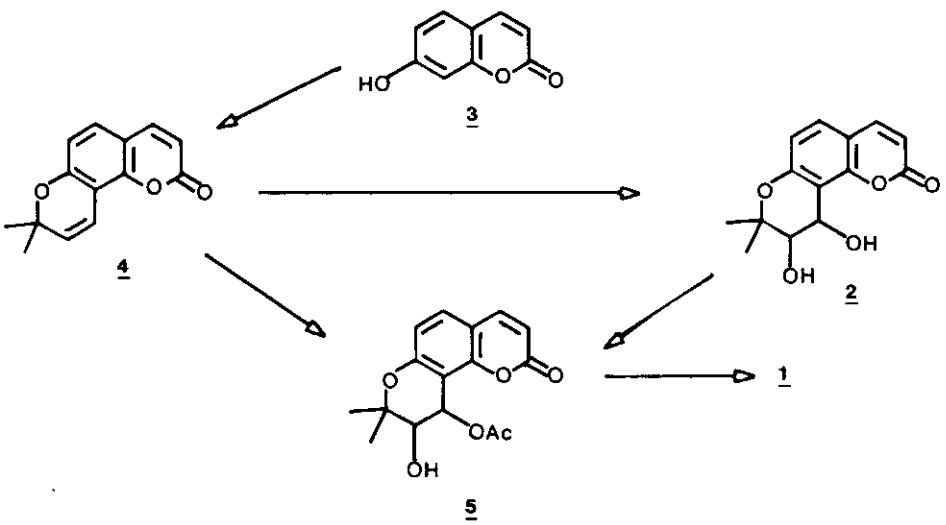
Earlier syntheses[4,5] of 3',4'-diacyl derivatives of cis diol 2 involve lengthy reaction pathways or depend on the isolation of pyranocoumarin compounds from plants as starting materials. Our efforts have been directed at the development of an efficient, practical and relatively simple scheme for the production of multigram amounts of various intermediates.

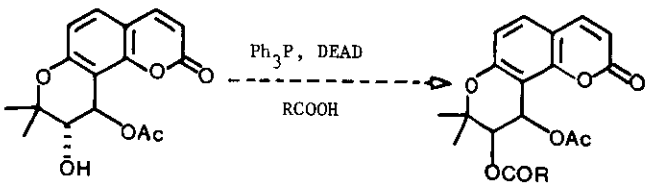
The most direct approach to 1 we envisaged is outlined in Scheme I. We planned to arrive at the key intermediate 5 if possible directly from seselin (4) by a modification of Woodward's hydroxylation procedure[6], or in two steps, one of which would require a regiospecific monoacetylation of diol 2. The introduction of the sensitive angeloyl group[7] was to be the final step in the sequence.

Scheme I



Umbelliferone (3) was converted into seselin (4) according to the literature procedure[8]. Treatment of 4 with iodine, silver acetate and "wet" acetic acid (1 equiv. water)[6] followed by heating at 90-95°C for several hours did not result in the formation of hydroxy acetate 5[9]. On adding excess water (ca. 19 equiv), the above reaction proceeded to completion providing a crystalline compound identified not as the desired cis hydroxy acetate 5 but as the trans hydroxy acetate 6 by NMR and comparison of melting point[9] (see experimental). Having obtained trans hydroxy acetate 6 in 63% yield, we hoped that application of the Mitsunobu[10] reaction (Scheme II) would enable us to directly get to a cis diacyl derivative such as praeruptorin A. In a model study, treatment of 6 with senecioic acid under the Mitsunobu conditions did not lead to product formation. The starting material was recovered unchanged.

Scheme II



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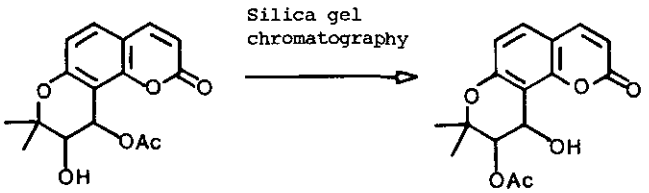
Our direct approach to the cis hydroxy acetate 5 having failed, we resorted to the route via diol 2. Cis-hydroxylation of seselin (4) has been reported using osmium tetroxide stoichiometrically[5b]. In our hands the workup proved to be cumbersome and yields were also low. Applying the more recently reported catalytic osmium tetroxide hydroxylation procedure[11] using a stoichiometric amount of N-methylmorpholine N-oxide provided diol 2 in 75% yield. Conversion of diol 2 to key intermediate 5 required a regiospecific monoacetylation at the 4'-hydroxy group. Conventional reagents such as acetyl chloride/benzene or acetic anhydride/pyridine gave mixtures containing compounds with 3' and/or 4'-acetoxy groups. Finally we succeeded in obtaining compound 5 when diol 2 was treated with acetic anhydride in THF in presence of BF_3-etherate[12] to effect regiospecific monoacetylation at the 4'-position. The gem-dimethyl groups in case of the cis hydroxy acetate 5 appear at the same frequency in the NMR spectrum, whereas in case of the trans hydroxy acetate 6 the signals arising from the two methyl groups are separated by 0.08 ppm[13].

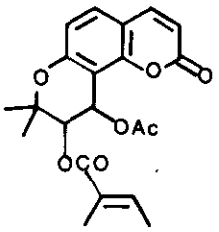
We encountered some problems in the large-scale purification of 5. The major drawback was that compound 5 was accompanied by much material resulting from polymerisation of THF[14]. Separation of the polymer proved to be difficult because of the susceptibility of compound 5 to undergo change on chromatography. The NMR of the product before chromatography had signals at δ3.98 and δ6.36 due to the 3' and 4' protons respectively. After chromatography, two new signals appeared at δ5.16 and δ5.37 pointing most probably to the formation of compound 7 by acetyl migration on the column. No attempts were made to isolate 7. The problem was finally solved by a quick flash chromatography[15] of the product mixture which provided pure compound 5 free from the polymer and from the rearranged product. We tried substituting THF with other solvents like dichloromethane, ether,



5 7

dioxane, and ether-THF mixtures. As expected, polymer formation was prevented, but the site selectivity for acetylation was also lost.

Having the cis hydroxy acetate 5 in hand, the stage was set for introducing the angeloyl group in the final step in the synthesis. This proved to be quite a challenge. The extreme sensitivity of the angeloyl group to undergo isomerisation about the double bond is well documented in the literature by Beeby[7]. We applied Beeby's conditions for formation of angelate esters (angeloyl chloride/THF/room temperature) to compound 5. The compound we isolated however was the tiglate 8 as judged by NMR which showed the vinylic proton at δ 6.75 (the corresponding proton in 1 appears at δ 6.01). Following a report for esterification under virtually neutral conditions we treated 5 with angelic acid/N,N-dimethylphosphoramic dichloride[16]. The starting material was recovered unchanged. Finally, treatment of compound 5 with angelic acid, DCC and 4-pyrrolidinopyridine (catalytic) in dichloromethane[17], afforded a mixture shown to contain (±)-praeruptorin A (= Pd-Ia) (1) and tiglate 8 in ca. 6% and 80% yields respectively based on recovered starting material (see experimental). Careful chromatography of the product mixture followed by semi-preparative HPLC afforded a sufficient amount of pure compound 1 for confirming its identity by direct comparison (GLC, HPLC, NMR, TLC, IR, mp) with an authentic sample kindly provided by Professor Shibata[18]. The tiglate 8 was also obtained directly by treating compound 5 with tiglic acid under the above conditions.



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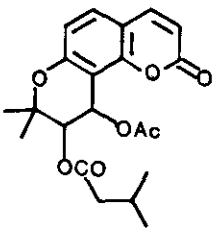

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The synthesis of another natural product (±)-dihydrosamidine (9) was accomplished with relative ease by treatment of hydroxy acetate 5 with isovaleric acid, DCC and 4-pyrrolidinopyridine at room temperature in a yield of 95%, a marked improvement over the earlier reported yield[5a]. The NMR and IR data and melting point were identical with the reported data[5a].

An interesting sidelight of this project was the application of the acylation reaction (DCC, 4-pyrrolidinopyridine, R-COOH) to the preparation of various monoacyl and diacyl derivatives of cis-khellactone (2).

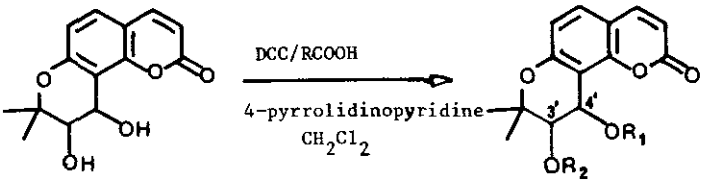
Treatment of 2 with different acids in presence of DCC and catalytic 4-pyrrolidinopyridine in dichloromethane gave a mixture of 4'-monoacylated and diacylated compounds (see Table I) which could be readily separated by chromatography[19]. No trace of the 3'-monoacylated compounds could be detected. On using excess acid and longer reaction times, the diacylated compound could generally be obtained as the major product.

The selectivity observed in the above reaction and the mild reaction conditions should make this a very useful tool in obtaining 4'-monoesters and 3',4'-diesters of cis-khellactone.

In summary, we have achieved the first synthesis of (±)-praeruptorin A (= Pd-Ia), albeit in a low yield in the final step owing to the instability of the angeloyl group. The intermediates have been obtained in high yields with a flexibility for synthesising many derivatives. A new method has been developed for the regiospecific formation of 4'-monoesters of cis diol 2. The approach can be applied to the synthesis of a host of pyranocoumarin natural products such as samidin, visnadin and suksdorfin[20].

Table I

Acylation of diol 2



2 10 – 14

Compound No.	R_1	R_2	Molar equivalents of RCOOH	Time	Yield[a] %	mp[b]
10	$-COCH_3$	$-COCH_3$	3.0	28 h[c]	95	177-179 °C
11	$-COCH_2CH(CH_3)_2$	H	2	55 min[c]	35[e]	164-166 °C
12	$-COCH_2CH(CH_3)_2$	$-CO-CH_2-CH(CH_3)_2$	6	18 h[d]	100	126-127 °C
13	$-COCH=C(CH_3)_2$	H	3.3	46 h[c]	38	148-150 °C
14	$-COCH=C(CH_3)_2$	$-COCH=C(CH_3)_2$			32	130-132 °C

(a) the yields are not optimised. (b) satisfactory NMR, IR and analytical data were obtained for all five compounds listed in the table. (c) room temperature (d) reflux

(e) 12% of 12 was also isolated.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL-90-Q or a Varian T-60 spectrometer. Chemical shifts are reported in δ units downfield from the internal standard tetramethylsilane. The coupling constants (J) are in Hertz. IR spectra were recorded on a Perkin-Elmer 157 spectrometer and are reported in reciprocal centimetres (cm^{-1}).

GLC analysis was performed on a Perkin Elmer 900 gas chromatograph equipped with a flame ionisation detector using a 3% OV-1 column. HPLC analysis was carried out on a Waters M-6000 A solvent delivery system with a μ porasil column, a U-6-K injector and a variable wavelength Knauer detector.

Tiglic acid was purchased from Fluka AG. Flash chromatography was performed according to the method reported by Still[15]. Moist extracts were dried over anhydrous sodium sulfate.

(±)-trans-4'-Acetylkhellactone (=(±)-trans-10-Acetoxy-9,10-dihydro-9-hydroxy-8,8-dimethyl-2H,8H-benzo [1,2-b:3,4-b'] dipyran-2-one)[21] (6) : To a stirred solution of seselin (4)[8] (0.5 g, 2.2 mmol) in glacial acetic acid (8 ml) was added silver acetate (0.93 g, 5.6 mmol). To this suspension powdered iodine (0.50 g, 2.3 mmol) was added over a period of 1.5 h and the reaction mixture was stirred for 30 min. Then water (0.69 g, 38 mmol) was added and the reaction mixture heated at 95-100°C for 25 h. After cooling to room temperature, the reaction mixture was filtered and the residue washed with hot benzene. The filtrate was concentrated on a rotary evaporator, diluted with water and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and water to give a fluffy solid (0.790 g) which was crystallised from benzene to give 6 (0.131 g), mp 196-198°C (lit.[9] mp 196-197°C). The mother liquor was concentrated and the residue chromatographed on florisil to give 0.164 g of 6 in an overall yield of 63%.
IR (KBr) : 1600, 1700, 3440; ^{1}H NMR ($CDCl_3$): 7.60 (1H, d, J=9.67, H-C(4)), 7.34 (1H, d, J=8.35, H-C(5)), 6.76 (1H, d, J=8.35, H-C(6)), 6.20 (1H, d, J=9.67, H-C(3)), 6.15 (1H, d, J=5.28, H-C(4')), 3.90 (1H, br t like collapsing to doublet on D_2O exchange, J=5.28, H-C(3')), 3.11 (1H, br d, OH, exchanging with D_2O), 2.19 (3H, s, $-OCOCH_3$), 1.47 and 1.39 (each 3H, s, 2 x CH_3-C(2')).

(±) cis-Khellactone (2) : Seselin[8] (4) (0.228 g, 1 mmol) was added to a solution of osmium tetroxide (10 mg, 0.04 mmol) and N-methylmorpholine-N-oxide dihydrate[11] (0.165 g, 1.1 mmol) in t-BuOH-THF-H_2O (10:3:1, 10 ml) and the reaction mixture stirred at room temperature for 3 d. Saturated $NaHSO_3$ solution (80 ml) was added, the mixture stirred at room temperature for 1 h and extracted with CH_2Cl_2 (2 x 40 ml) to give a solid (0.216 g) which was purified by chromatography on florisil. Elution with $CHCl_3$ gave unreacted seselin (4) (0.053 g), while further elution with 4% CH_3OH-$CHCl_3$ provided 2 (0.150 g, 75%, based on recovered starting material) which was purified by crystallisation from EtOAc-petroleum ether, mp 159-161°C (lit.[5b] mp 159-161°C); IR (KBr) : 1600, 1680, 1720, 3400; ^{1}H NMR ($CDCl_3$) : 7.64 (1H, d, J=10.04, H-C(4)), 7.30

(1H, d, J=8.5, H-C(5)), 6.76 (1H, d, J=8.5, H-C(6)), 6.22 (1H, d, J=10.04, H-C(3)), 5.19 (1H, br t like collapsing to doublet on D_2O exchange, J=5.40, H-C(4')), 4.26 (1H, br d exchanging with D_2O, OH), 3.86 (1H, t like collapsing to doublet on D_2O exchange, J=5.40, H-C(3')), 3.30 (1H, d, J=6.48, exchanging with D_2O, OH), 1.46 and 1.40 (each 3H, s, 2 x CH_3-C(2')).

(±)-cis-4'-Acetylkhellactone (5) : A solution of freshly distilled $BF_3.Et_2O$ (0.11 ml, 0.74 mmol) and Ac_2O (0.121 mg, 1.19 mmol) in dry THF (3 ml) was added over a period of 2 min to a solution of 2 (0.210 g, 0.7 mmol) in 6 ml of THF. After being stirred at room temperature for 2.8 h the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (2 x 60 ml). The $CHCl_3$ extract was washed with water, dried and concentrated to give a viscous oil (1.1 g), which crystallised from benzene-petroleum ether to give 0.149 g (65%, based on recovered starting material) of the known compound 5[4,5] as a white solid, mp 189-191°C (lit.[4] mp 192°C); IR (KBr) : 1595, 1700, 1725, 3460; ^1H NMR ($CDCl_3$) : 7.55 (1H, d, J=10.20, H-C(4)), 7.28 (1H, d, J=8.40, H-C(5)), 6.73 (1H, d, J=8.40, H-C(6)), 6.36 (1H, d, J=4.86, H-C(4')), 6.18 (1H, d, J=10.20, H-C(3)), 3.98 (1H, br d collapsing to doublet on D_2O exchange, J=4.86, H-C(3')), 2.66 (1H, br s exchanging with D_2O, OH), 2.22 (3H, s, $-OCOCH_3$), 1.46 (6H, s, 2 x CH_3-C(2')).

Direct crystallisation of 5 could not be repeated in scale-up batches. A quick flash chromatography[15] of the product mixture followed by crystallisation provided pure compound 5.

(±)-cis-4'-Acetyl-3'-tigloylkhellactone (8) : To a solution of 5 (0.076 g, 0.25 mmol) in dry CH_2Cl_2 (30 ml) were added 4-pyrrolidinopyridine (2 mg, 0.014 mmol), tiglic acid (0.080 g, 0.8 mmol) and DCC (0.260 g, 1.26 mmol) and the reaction mixture refluxed for 125 h. Starting material was still present as seen by TLC. The reaction mixture was then cooled, and the separated urea filtered off and washed with CH_2Cl_2. The filtrate was concentrated and purified by flash chromatography to give compound 8, 45 mg (86%, based on recovered starting material) which crystallised from cyclohexane, mp 147-149°C; IR (KBr) : 1610, 1705, 1715, 1735; ^1H NMR : 7.52 (1H, d, J=9.36, H-C(4)), 7.28 (1H, d, J=8.64, H-C(5)), 6.91-6.64 (1H, m, $CH_3C=\underline{CH}CH_3$), 6.73 (1H, d, J=8.64, H-C(6)), 6.52 (1H, d, J=4.68, H-C(4')), 6.16 (1H, d, J=9.36, H-C(3)), 5.33 (1H, d, J=4.68, H-C(3')), 2.09 (3H, s, $-OCOCH_3$), 1.82 (3H, s, $\underline{CH_3}C=CHCH_3$), 1.78 (3H, br d, J=6.12, $CH_3C=CH\underline{CH_3}$), 1.46 and 1.41 (each 3H, s, 2 x CH_3-C(2')). Anal. Calcd. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found : C, 65.46; H, 5.68.

(+)-Praeruptorin A (= Pd-Ia) (1) : To a stirred solution of 5 (0.152 g, 0.5 mmol) in dry CH_2Cl_2 (40 ml) were added 4-pyrrolidinopyridine (4 mg, 0.03 mmol), angelic acid[22] (0.100 g, 1 mmol) and DCC (0.312 g, 1.52 mmol) and the reaction mixture refluxed for 29 h. A work-up procedure similar to that for compound 8 provided a viscous oil (0.55 g). Flash chromatography[15] (25% EtOAc-$CHCl_3$) of the oil gave one fraction (27 mg) which by GLC and HPLC was found to contain praeruptorin A (1)[18] together with the tiglate ester 8 in a ratio of 1:3 (6% yield of 1 based on recovered starting material). Subsequent column fractions afforded compound 8 (0.120 g) associated with some impurities, and unreacted starting material 5 (0.052 g). Semi-preparative HPLC of the fraction containing compound 1 gave 1.5 mg of 1 as a white solid, mp 145-147.5°C[23]; IR (KBr) : 1492, 1573, 1604, 1720-1760; ^{1}H NMR ($CDCl_3$) : 7.53 (1H, d, J=10.08, H-C(4)), 7.28 (1H, d, J=8.60, H-C(5)), 6.74 (1H, d, J=8.60, H-C(6)), 6.54 (1H, d, J=4.84, H-C(4')), 6.16 (1H, d, J=10.08, H-C(3)), 6.07 (1H, m, $CH_3C=\underline{CH}CH_3$), 5.35 (1H, d, J=4.84, H-C(3')), 2.11 (3H, s, $-OCOCH_3$), 1.94 (3H, br d, $CH_3C=CH\underline{CH}_3$), 1.88 (3H, br s, $C\underline{H}_3C=CHCH_3$), 1.47 and 1.43 (each 3H, s, 2 x CH_3-C(2')). Comparison (mixed mp[23], TLC, HPLC, GLC, IR, NMR) of compound 1 with an authentic sample[18] established its identity as (+)-praeruptorin A (= Pd-Ia).

General Procedure for the Preparation of Monoacyl and Diacyl Derivatives (10-14) of cis-Khellactone (2). (Table I) : To a stirred solution of diol 2 (1 mmol) in CH_2Cl_2 (25-50 ml) were added 4-pyrrolidinopyridine (0.2 mmol), DCC (2-5 mmol) and carboxylic acid (2-5 mmol) and the mixture stirred at room temperature or refluxed for the appropriate time. The separated urea was filtered off and the filtrate concentrated. The material thus obtained was chromatographed on silica gel according to the method of Still[15] or on florisil (60-100 mesh) and the pure compounds 10-14 obtained by crystallisation.

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18. We thank Professor S. Shibata, Department of Pharmacognosy and Phytochemistry, Meiji College of Pharmacy, Tokyo, Japan, for sending us a sample of (+)-praeruptorin A (= Pd-Ia) for comparison.

19. This method could not be utilised for the preparation of cis-4'-acetylkhellactone (5), because of its susceptibility to undergo change on chromatography.

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23. The melting point of compound 1 was not depressed on admixture with an authentic sample[18] of (+)-praeruptorin A (= Pd-Ia) which melted at 144.5-147°C.

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