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CALANONE, A NOVEL COUMARIN FROM *Calophyllum teysmannii*

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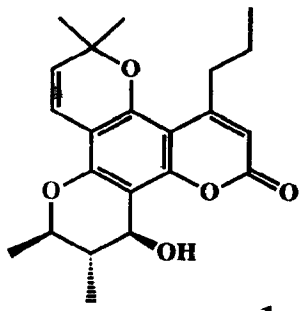
Abstract: During a survey of latex samples of *Calophyllum teysmannii* for anti-HIV coumarins (calanolide A, costatolide), calanone (5), an unprecedented benzoyl substituted coumarin, was isolated and its structure determined by spectroscopic analyses. The known soulattrolide (3), and the related ketone 4 were also isolated. Soulattrolide inhibited the cytopathic effect of *in vitro* HIV-1 infection, while calanone (5) and the ketone 4 were inactive.

Following the discovery of the HIV-inhibitory activity of calanolide A (1),[1] isolated from leaves and twigs of *Calophyllum lanigerum* var. *austrocoriaceum*, and its subsequent identification as a new subclass of non-nucleoside HIV-1 specific reverse transcriptase inhibitor,[2] we set out to establish an adequate supply of 1 for preclinical development. Our strategy focused on the latex scraped from small wounds in the bark as a sustainable resource of the desired coumarins. As reported,[3] we have not yet identified such a source of calanolide A, but did find that latex from *C. teysmannii* var. *inophylloide* contained large quantities of costatolide (2) and soulattrolide (3), which also exhibit anti-HIV activity. As we continued to survey specimens of *C. teysmannii* for these compounds, we uncovered a few trees of this species which contained a different coumarin mixture. From these we have isolated a unique new coumarin, calanone, the subject of this report.



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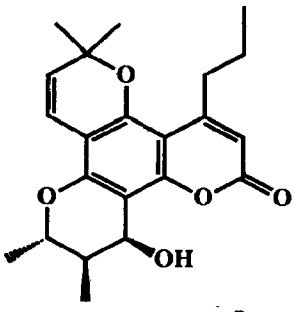

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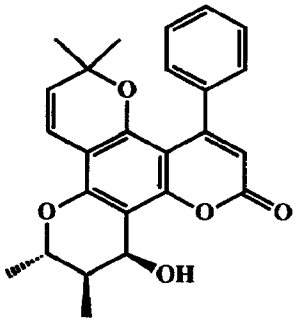

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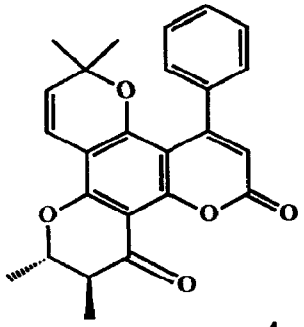
Latex samples from several dozen trees of *C. teysmannii* were extracted by trituration with CH_2Cl_2-CH_3OH (1:1). As noted earlier,[1,3] the prenylated coumarins like calanolide A (1) or costatolide (2) give a very characteristic stain on TLC plates sprayed with a vanillin/H_2SO_4 reagent; an intense deep blue spot develops over a 24 hour period. All the latex extracts we have obtained from *C. teysmannii* have been active in an anti-HIV assay,[4] and most gave a TLC profile matching that of our original sample, which contained **2** and **3**.[3] Latex extracts from three specimens of *C. teysmannii* var. *inophylloide* (Kadushin & Othman 1354, 1361 and 1432), however, gave a different TLC profile, indicating the presence of **3** and two slightly less polar coumarins. HPLC of this extract (silica gel, hexane-EtOAc, 7:3) provided soulattrolide, **3** (~ 15% of the extract), the related ketone **4** (~2.5%) and calanone, **5** (~ 45%). Compounds **3** and **4** were identified by comparison of their spectral data with those published earlier.[5,6]



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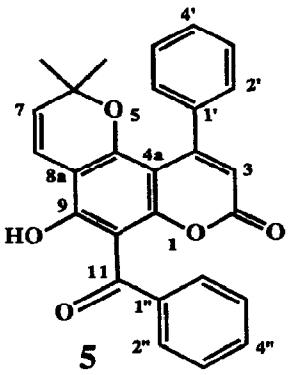

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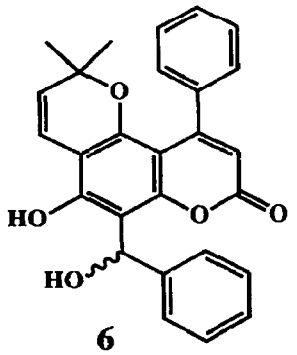

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Calanone (**5**), a pale yellow glass, analyzed for $C_{27}H_{20}O_5$ by HRFABMS.[7] Absorption bands in the IR spectrum were appropriate for hydroxyl (3455 cm^{-1}) and carbonyl (1738 cm^{-1}) functionalities. Comparison of the ^1H NMR and ^{13}C NMR spectra of calanone (**5**) with those recorded for soulattrolide (**3**), revealed significant structural similarities. Characteristic ^1H NMR and ^{13}C NMR resonances for the substituted coumarin ring system, a phenyl substituent and a 2,2-dimethylchromene system were apparent (see Table 1). HMQC and HMBC correlations allowed assignment of the carbon resonances and the regiochemical placement of substituents about the coumarin moiety. A correlation from the phenyl C1' (δ 139.7) to H3 confirmed that a phenyl group was attached to C4, as in soulattrolide, while a correlation from δ 102.4 to H3 allowed assignment of C4a. C8a (δ 105.7) was defined by a correlation to H7, and correlations from C8a, C9 (δ 161.5) and C10 (δ 103.8) to a phenolic proton placed the phenol group at C9. NMR resonances assigned to the 2,3-dimethylchromanol ring in **1-4** were absent in the spectra of calanone. Instead, a benzoyl moiety attached at C10 was suggested by ^{13}C NMR resonances of a conjugated ketone (δ 198.8) and six additional aromatic carbons. The C9 phenol proton showed evidence of being hydrogen-bonded, since it appeared as a sharp singlet in the ^1H-NMR at δ 12.46. This is consistent with strong hydrogen bonding between the phenol and a C10 benzoyl keto-group; this would also explain the intense IR absorption at 1607 cm^{-1}. We were thus able to assign structure **5** for calanone. To our knowledge, this is the first prenylated coumarin substituted with a benzoyl group at position 8 on the coumarin nucleus.

TABLE 1.

^{13}C and ^{1}H-NMR spectral data for calanone (**5**)

Carbon #	δ ^{13}C[a]	δ ^{1}H[b]	HMBC Correlations
2	158.0[c]		
3	112.6	5.88, s	C4a, C1′
4	155.3[c]		
4a	102.4		
4b	156.4		
6	79.1		
7	127.0	5.42, d, J = 10.5 Hz	C6, C8a
8	115.2	6.64, d, J = 10.5 Hz	C4b, C6, C9
8a	105.7		
9	161.5		
10	103.8		
10a	155.9[c]		
11	198.8		
12	27.4	0.98 (3H), s	C6, C7
13	27.4	0.98 (3H), s	C6, C7
1′	139.7		
2′	127.2	7.23 (2H), m	C3′
3′	127.5	7.37 (2H), m	C4′
4′	127.8	7.37, m	
1″	140.3		
2″	128.2	7.65 (2H), dd, J = 8.0, 1.7 Hz	C3″
3″	128.2	7.46 (2H), t, J = 8.0 Hz	C1″, C2″
4″	132.3	7.57, tt, J = 8.0, 1.7 Hz	C2″, C3″
OH		12.46, s	C8a, C9, C10

[a] 125 MHZ, $CDCl_3$
[b] 500 MHz, $CDCl_3$
[c] Resonances may be interchangeable

In vitro XTT-based anti-HIV-1 evaluation[4] of the coumarins isolated from *C. teysmannii* var. *inophylloide* demonstrated that calanone (**5**) and the ketone **4** were inactive. It was previously observed for calanolide A (**1**) and others in this class of coumarin that the presence of a benzylic alcohol at C10 (C11 in calanone) was important for anti-HIV activity.[1,2] With this in mind, we treated calanone with $NaBH_4$ to generate the benzylic alcohol **6**.[8] The resulting racemic product was assayed against HIV-1, but was also inactive.

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7. **5**: HRFABMS *m/z* 425.1366 (MH^+, calcd 425.1311 for $C_{27}H_{21}O_5$); IR (film) ν_{max} 3455, 3065, 2979, 2921, 1738, 1607, 1597, 1571, 1442, 1360, 1270, 1135 cm^{-1}; UV λ_{max} (MeOH) 242 nm (ϵ = 14,800), 274 (20,900), 320 (10,300); ^1H NMR and ^{13}C NMR see Table 1.
8. **6**: EIMS *m/z* 426, appropriate for $C_{27}H_{22}O_5$; ^1H NMR ($CDCl_3$) δ 0.91 (3H, s), 0.92 (3H, s), 5.28 (1H, s), 5.36 (1H, d, J = 10.0 Hz), 5.87 (1H, s), 6.60 (1H, d, J = 10.0 Hz), 7.19 (2H, m), 7.35 (6H, m), 7.56 (2H, dd, J = 9.0 and 1.5 Hz), 9.79 (1H, s).

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