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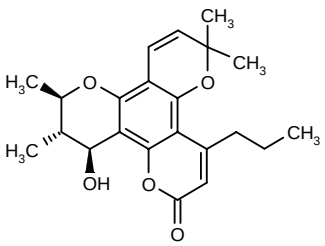
CCC: 0377-8282/99

Calanolide A

Antiviral for AIDS
Reverse Transcriptase Inhibitor

NSC-675451
NSC-664737 (as racemic)

(+)-(10*R*,11*S*,12*S*)-12-Hydroxy-6,6,10,11-tetramethyl-4-propyl-11,12-dihydro-2*H*,6*H*,10*H*-benzo[1,2-*b*:3,4-*b*':5,6-*b*'']-tripyran-2-one



$C_{22}H_{26}O_5$ Mol wt: 370.4424

CAS: 142632-32-4
CAS: 151005-68-4 (as racemic)
CAS: 163661-45-8 [as (–)-isomer]

EN: 204331
EN: EN:217623

Isolation

Calanolide A has been isolated from dried fruits and twigs from *Calophyllum lanigerum* var. *Austrocoriaceum*. The material was ground and percolated with a 1:1 mixture of dichloromethane and methanol; the organic solution was evaporated under vacuum and the dried residue was submitted to a solvent-solvent partitioning protocol. Finally, the active fractions were purified by vaccum liquid chromatography and the (+)-enantiomer was isolated (1, 2).

Synthesis

Calanolide A can be synthesized by several different ways:

1) The cyclization of phloroglucinol (I) with butyrylacetic acid ethyl ester (II) by means of concentrated sulfuric acid gives 5,7-dihydroxy-4-propyl-2*H*-1-benzopyran-2-one (III), which is condensed with propionyl chloride by means of $AlCl_3$ in nitrobenzene, yielding the 8-propionyl derivative (V). The cyclization of (V) with 3-hydroxy-3-methylbuytyraldehyde dimethylacetal (VI) in refluxing pyridine affords the benzodipyran (VII), which is cyclized again with paraldehyde or acetaldehyde dimethylacetal by means of *p*-toluenesulfonic acid and trifluoroacetic acid in pyridine at 140 °C to give a mixture of the diastereomeric racemates (VIII) and (IX), which are separated by column chromatography. The reduction of racemate (VIII) with $NaBH_4$ /$CeCl_3$ in ethanol yields a new mixture of the racemic hydroxy epimers (X) (racemic calanolide A) and (XI), which are separated by semipreparative HPLC (3-5). Racemic calanolide A (X) is finally submitted to optical resolution by semipreparative chiral HPLC (4-6). Scheme 1.

2) The condensation of 5,7-dihydroxy-4-propyl-2*H*-1-benzopyran-2-one (III) with *N*-methylformanilide (XII) by means of $POCl_3$ in hot dichloromethane gives the carbaldehyde (XIII), which is cyclized with 3-chloro-3-methyl-1-butyne (XIV) by means of $ZnCl_2$/K_2CO_3 in hot 2-butanone/DMF, yielding the benzodipyran-carbaldehyde (XV). The enantioselective reaction of (XV) with 2(*E*)-butene and the chiral borane (+)-(*E*)-crotyldiisopinocamphenylborane (XVI) affords the single (*R*,*R*)-enatiomer (XVII). The selective silylation of (XVII) with TBDMS-Cl as usual gives the monosilyl ether (XVIII), which is cyclized by means of mercuric acetate and $NaBH_4$ in THF, yielding the silylated (10*R*,11*R*,12*R*)-benzotripyran (XIX). The desilylation of (XIX) with tetrabutylammonium fluoride in THF gives the (10*R*,11*S*,12*R*)-benzotripyran (XX) (calanolide B), which is converted into calanolide A by inversion of the C-12 OH-group carried out with a modified Mitsunobu reaction with dimethyl azodicarboxylate (DEAD)/trimethylphosphine/chloroacetic acid in toluene/THF, followed by treatment with NH_4OH in methanol (7, 8). Scheme 2.

3) Synthesis of *ent*-Calanolide A, (–)-calanolide A: The Clemensen reduction of 5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran-4-one (XXI) with Zn/HCl in methanol gives 5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran (XXII), which is condensed with methyl 2-hexynoate (XXIII) using the palladium catalyst Pd_2dba_3 to yield dihydrobenzodipyran (XXIV). The regio- and enantioselective allylic alkylation of (XXIV) with 2-methyl-2(*E*)-butenyl carbonate (XXV) catalyzed by Pd_2dba_3

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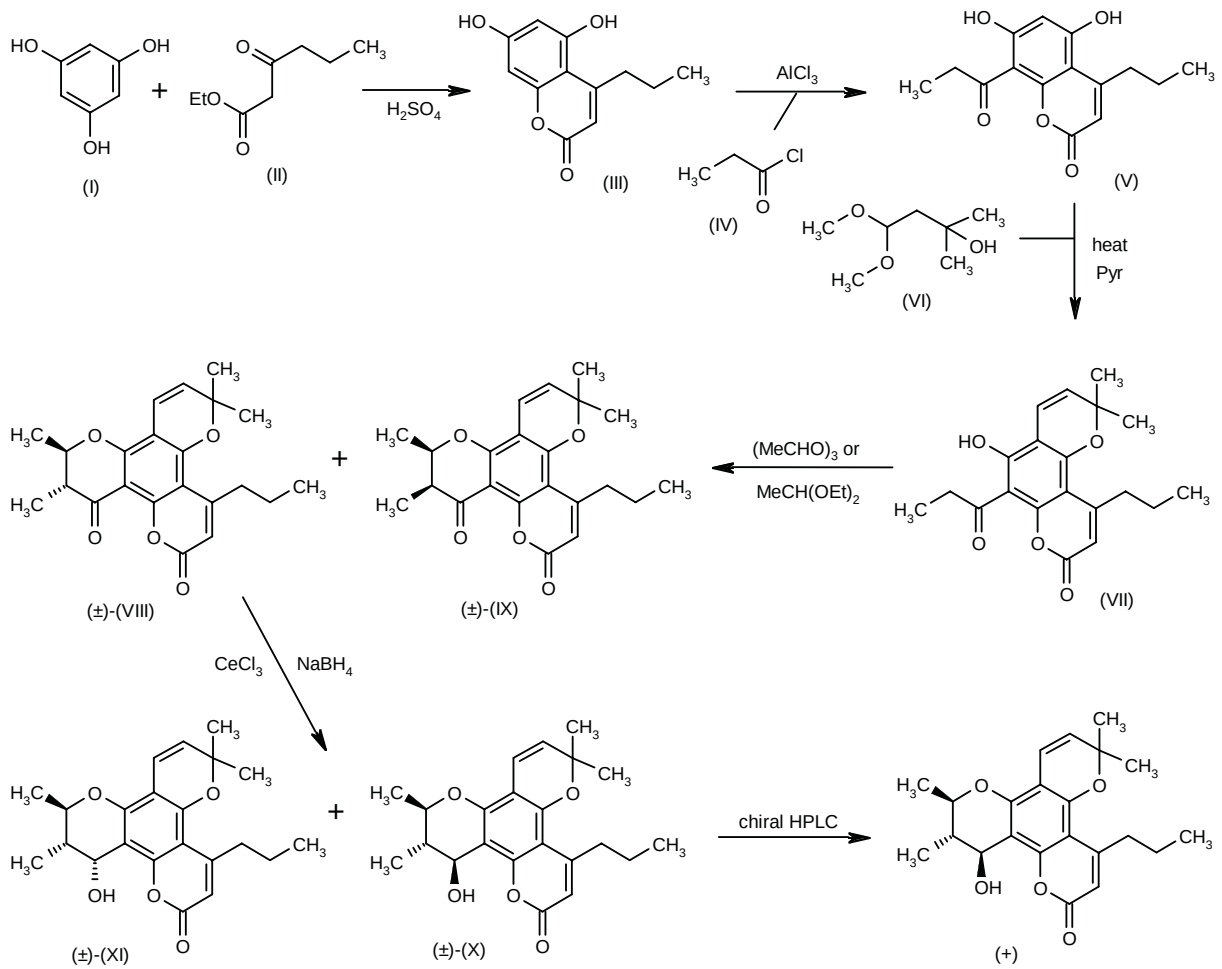
Scheme 1: Synthesis of Calanolide A

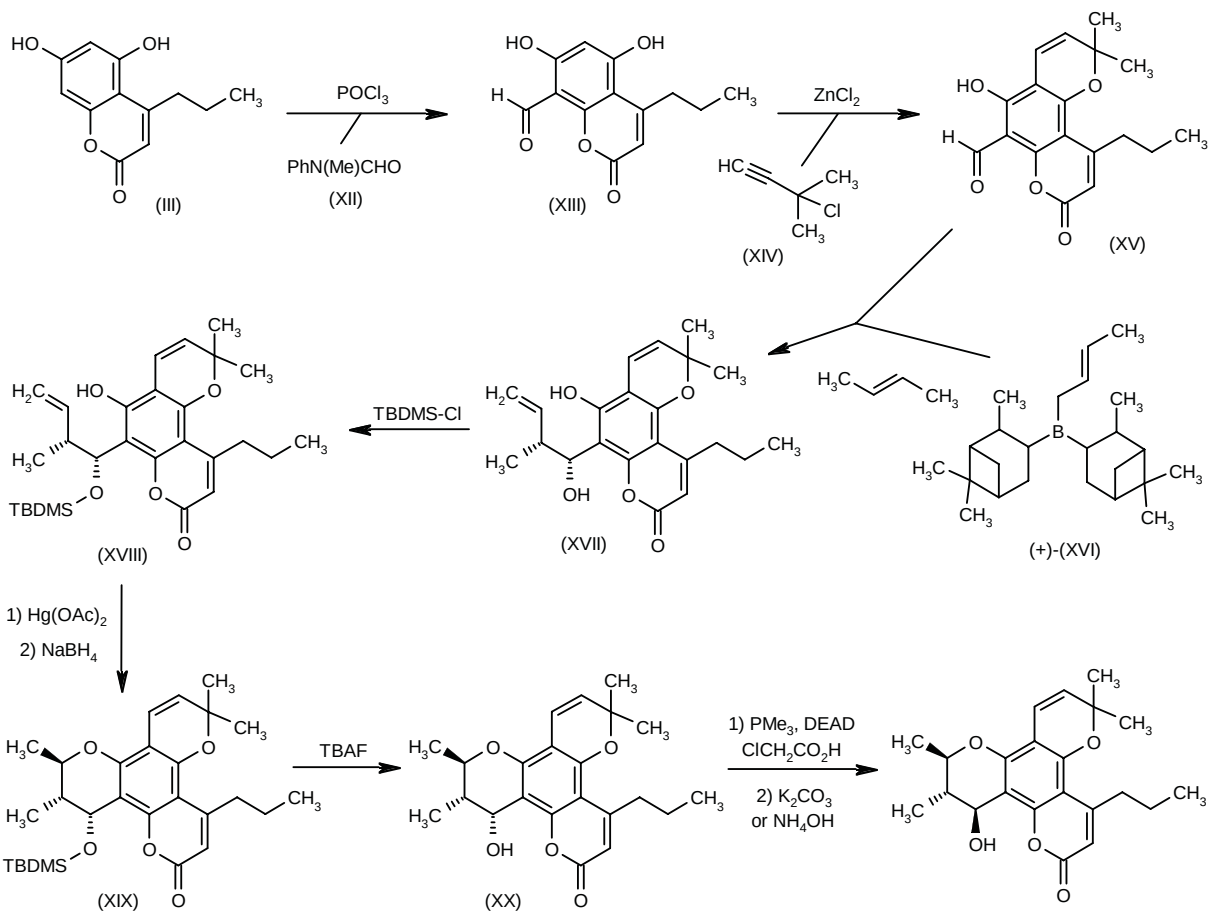


using a chiral ligand affords the chiral dihydrobenzodipyran ether (XXVI), which is dehydrogenated with dichlorodicyanobenzoquinone (DDQ) in dioxane to (XXVII). The chemoselective hydroboration of (XXVII) with 9-BBN and H_2O_2 gives the alcohol (XXVIII) with high diastereomeric selectivity. The Dess-Martin oxidation of (XXVIII) yields the expected aldehyde (XXIX), which is cyclized to (10*S*,11*R*,12*R*)-benzotripyran (XXX) (*ent*-calanolide B). Finally, this compound is converted into *ent*-calanolide A by inversion of the C-12 hydroxy group carried out with a modified Mitsunobu reaction with dimethyl azodicarboxylate (DEAD)/trimethylphosphine/chloroacetic acid in toluene/THF, followed by treatment with NH_4OH in methanol. The authors indicate that Calanolide A can be synthesized in the same way by simply using the mirror image of the chiral ligand in the addition of carbonate (XXV) to dihydrobenzodipyran (XXIV) (9). Scheme 3.

4) The aldol condensation of the previously described benzodipyran (VII) with acetaldehyde catalyzed by $TiCl_4$/lithium diisopropylamide (LDA) in heptane/THF/ethylbenzene gives a racemic mixture of the enantiomers (XXXI) and (XXXII), which is enzymatically resolved with lipase PS-30 or lipase-AK and vinyl acetate in *tert*-butyl methyl ether to yield a mixture of unchanged 3(*S*)-hydroxy-2(*R*)-methylbutyryl-enantiomer (XXXI) and acetylated enantiomer (XXXIII), which are easily separated by column chromatography. The cyclization of (XXXI) by means of triphenylphosphine and diethyl azodicarboxylate (DEAD) in THF gives the (10*R*,11*R*)-precursor (XXXIV), which is finally reduced with $NaBH_4$/triphenylphosphine oxide /$CeCl_3$ in ethanol and purified by chiral HPLC (10). Scheme 4.

5) Synthesis of [^{14}C]-labeled calanolide A: The aldol condensation of benzodipyran (VII) with [1,2-^{14}C]-labeled acetaldehyde catalyzed by $TiCl_4$/lithium diisopropylamide (LDA) in heptane/THF/ethylbenzene gives a racemic

Scheme 2: Synthesis of Calanolide A



mixture of enantiomers (XXXV) and (XXXVI), which is enzymatically resolved with lipase-AK and vinyl acetate in *tert*-butyl methyl ether to yield a mixture of unchanged 3(*S*)-hydroxy-2(*R*)-methylbutyryl enantiomer (XXXVI) and acetylated enantiomer (XXXVII), which are easily separated by column chromatography. The cyclization of (XXXVI) by means of triphenylphosphine and diethyl azodicarboxylate (DEAD) in THF gives the (10*R*,11*R*)-precursor (XXXVIII), which is finally reduced with $NaBH_4$/triphenylphosphine oxide /$CeCl_3$ in ethanol and purified by chiral HPLC (11). Scheme 5.

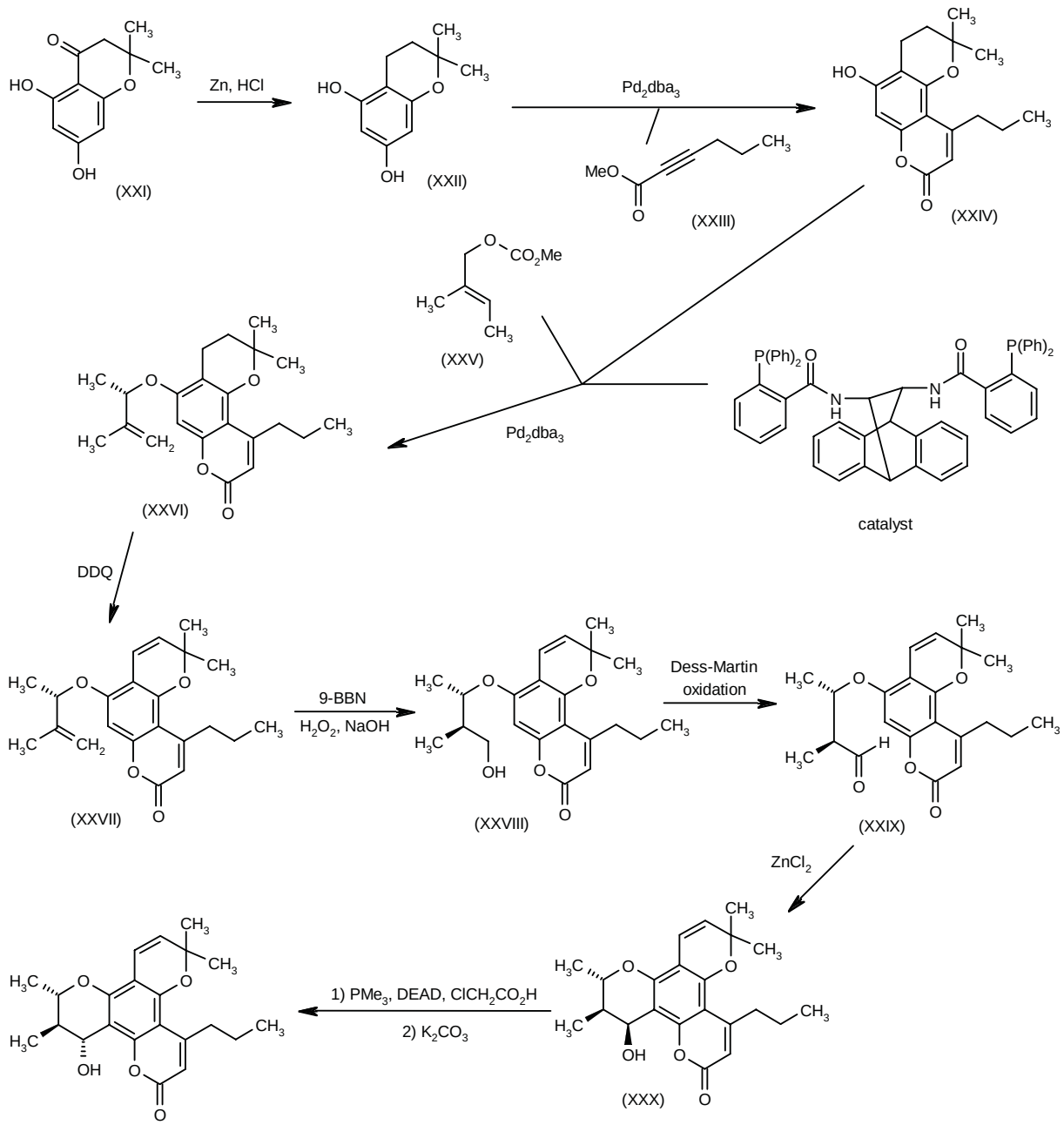
Description

Natural product: oil, $[\alpha]_D$ +60° (c 0.7, chloroform) (1, 2). Synthetic product: m.p. 47-50 °C, $[\alpha]_D^{25}$ +68.8° (c 0.7, chloroform) (4, 5); m.p. 45-8 °C, $[\alpha]_D^{20}$ +72° (c 0.51, chloroform) (7); m.p. 45-8 °C, $[\alpha]_D^{20}$ +66° (c 0.5, chloroform) (8); $[\alpha]_D^{25}$ +56.7 ° (c 0.73, chloroform) (10). Racemic: initial m.p. 52-4 °C, which is increased to 101-3 °C after thorough drying (3, 4).

Introduction

Since the identification that human immunodeficiency virus (HIV) was the cause of acquired immune deficiency syndrome (AIDS) a decade ago (12) and the discovery of azidothymidine, HIV chemotherapy has been dominated by nucleoside reverse transcriptase inhibitors (NRTIs) derived from 2',3'dideoxynucleoside (ddN), such as azidothymidine (AZT, Retovir®), didanosine (ddI, Videx®), zalcitabine (ddC, Hivid®), stavudine (d4T, Zerit®) and lamivudine (3TC, Epivir®). The HIV protease inhibitors, saquinavir (Invirase®), ritanovir (Norvir®), indinavir (Crixavan®) and nelfinavir (Viracept®), have also become available (13, 14). Although these agents can extend the life of an AIDS patient, none can cure the disease and all are associated with serious adverse effects. For instance,

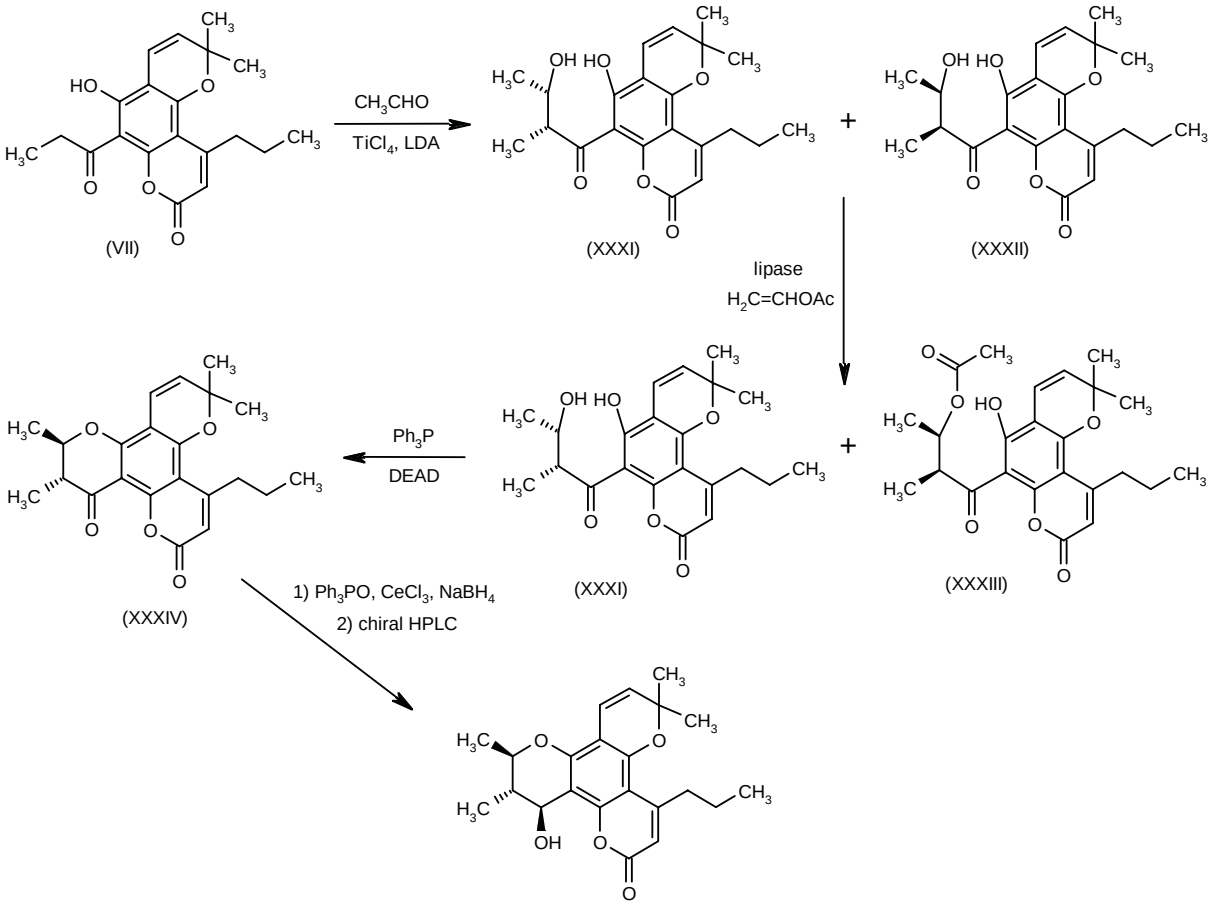
Scheme 3: Synthesis of *ent*-Calanolide A



suppression of bone marrow formation resulting in anemia and leukopenia accompanies AZT therapy and requires frequent blood transfusions (15); moreover, AZT has a short half-life and high doses (250 mg) must be taken every 4 h. Therapy with ddI, ddC and d4T causes painful sensory-motor peripheral neuropathy and acute pancreatitis and hepatotoxicity (16, 17). In addition to adverse effects, long-term treatment with all these agents has led to drug-resistant HIV strains, and primary infections can now occur with AZT-resistant HIV strains (18-21).

The need for new candidates with improved selectivity and activity for anti-HIV therapy is therefore evident. Through chemical synthesis and natural product screening, compounds have been identified which target different stages of HIV replication, such as the protease

Scheme 4: Synthesis of Calanolide A



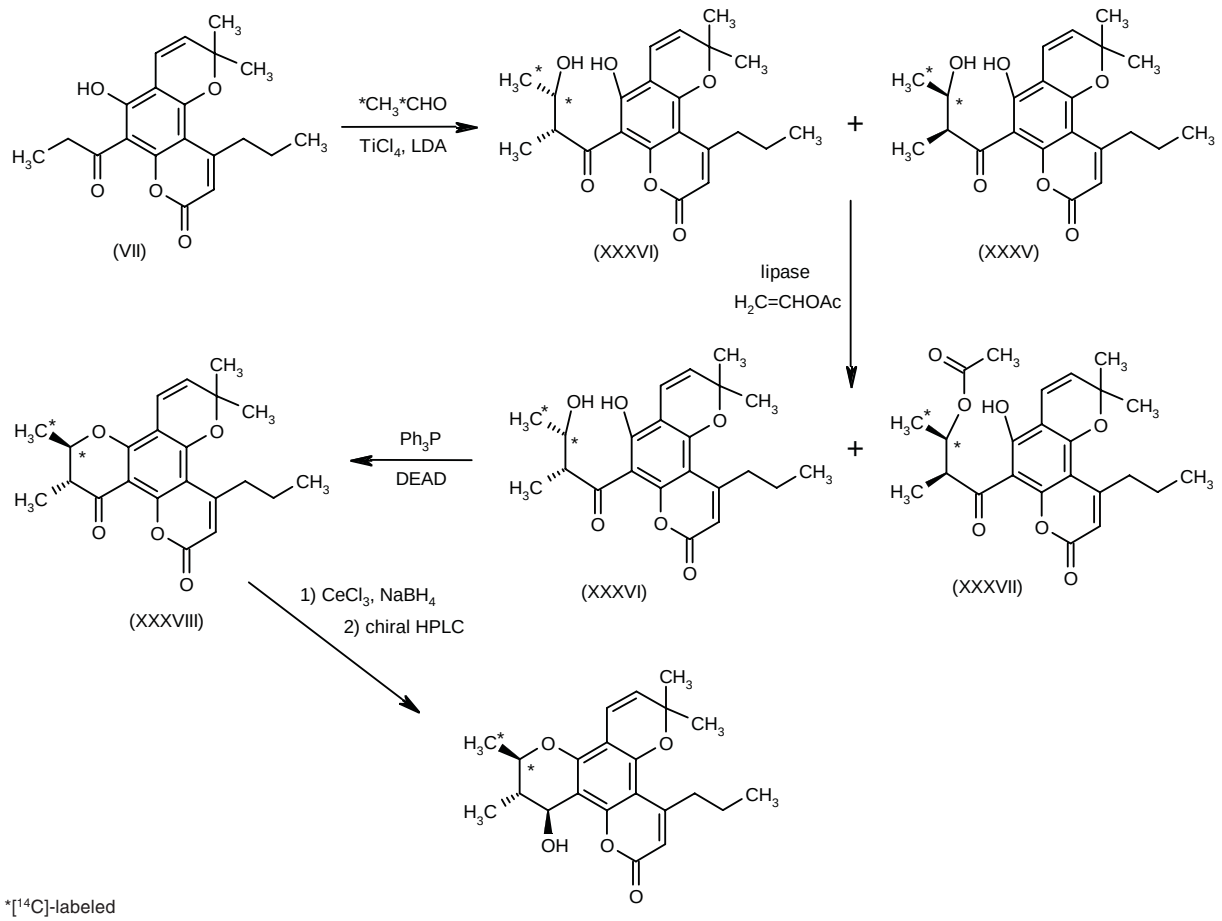
inhibitors mentioned above. Another important enzyme target for anti-HIV drugs is reverse transcriptase (22-24). Nonnucleoside reverse transcriptase inhibitors (NNRTIs) have begun to play an increasingly important role in HIV therapy (25-27). NNRTIs are highly specific and selective, targeting HIV reverse transcriptase and not cellular DNA or other DNA polymerases, which enables them to be relatively nontoxic to human cells. Several NNRTIs have been identified, including TIBO, nevirapine, pyridinone, BHAP, HEPT, TSAO and alpha-APA (25-28). The exact mechanism(s) of action of NNRTIs is unclear. However, the binding of these compounds appears to be slow, reversible and noncompetitive with template-primers and deoxynucleoside triphosphates. The binding site of nevirapine, TIBO and α-APA is suspected to be the hydrophobic pocket of HIV-1 (29-33). However, once again the emergence of strains of HIV resistant to NNRTIs is a major concern (27).

Second-generation HIV-1-specific NNRTIs have been identified from coumarin derivatives isolated from tropical plants (*Calophyllum*), with (+)-calanolide A, (–)-calanolide B and inophyllum B emerging as the most potent agents against several host lines (2, 4, 34-36). These agents are pharmacologically different from other established NNRTIs and are active against AZT- and pyridinone-resistant HIV-1 strains (37-39). Used in combination with other anti-HIV therapies, they may represent a novel approach to HIV treatment. In addition, these compounds are potential anti-HIV chemotypes for drug development.

Pharmacological Actions

The biological activity of (+)-calanolide A was examined *in vitro* with laboratory and clinical strains of HIV isolates. The agent completely inhibited both AZT-resistant strain G910-6 and pyridinone-resistant strain A17 (EC_{50} = 0.03 μM and 0.4 μM, respectively). Direct toxicity to cells occurred at a concentration approximately 100- to 200-fold the effective dose. Inhibition profiles demonstrating the specificity of (+)-calanolide A were also reported,

Scheme 5: Synthesis of [^{14}C]-Calanolide A



showing that the agent (200 mcg/ml) inhibited HIV-1 reverse transcriptase with no activity observed against HIV-2 reverse transcriptase or avian myeloblastosis virus. (+)-Calanolide A was effective in inhibiting HIV-1 reverse transcriptase DNA- and RNA-dependent DNA polymerases (IC_{50} = 0.38 and 0.32 μM, respectively). In addition, TIBO-resistant HIV-1 reverse transcriptase was inhibited by 91.5% (4). The pharmacological profiles of calanolide A and other NNRTIs are shown in Table I.

Other studies have also demonstrated that calanolide A actively inhibits a wide variety of other HIV-1 strains, including laboratory and promonocytotropic (EC_{50} = 0.1-0.17 μM) and lymphotropic isolates (EC_{50} = 0.15-0.47 μM). Further characterization using viral life cycle studies with infected CEM-SS target cells showed that calanolide A (5 μM) was protective within 6 h. However, when addition of calanolide A was delayed to 12 h or more, antiviral protection was reduced, indicating that the agent acts early in the infection process. The agent also dose-dependently inhibited recombinant HIV-1 reverse transcriptase (IC_{50} = 2.0 μM), with complete inhibition occurring with doses > 10 μM; no activity against cellular DNA polymerases or HIV-2 reverse transcriptase was observed at concentrations up to 200 μM. However, a calanolide A-resistant strain emerged after serial passage of the virus in host cells exposed to increasing concentrations of the agent; reverse transcriptase from this strain, although unaffected by calanolide A, retained sensitivity to other NNRTIs as well as nucleoside transcriptase inhibitors (40).

Kinetic analysis of the inhibition of HIV-1 reverse transcriptase by (+)-calanolide A showed that inhibition occurs at a competitive and a noncompetitive binding site. The agent appears to bind near the active site of the enzyme, interfering with deoxynucleoside triphosphate binding. In addition, (+)-calanolide A was shown to share some binding sites with phosphonoformic acid and 1-ethoxymethyl-5-ethyl-6-phenylthio-2-thiouracil, suggesting that it interacts with reverse transcriptase near the pyrophosphate binding site and the active site (41).

Further studies characterizing (+)-calanolide A activity have shown that it has a primary selection for virus strains

Table I: Pharmacological profile of selected NNRTIs (from Prous Science MFLine database).

Compound	RTI (IC_{50}, μM)	HIV-1 antiviral activity (IC_{50}, μM)	Anti HIV-1 (AZT-resistant, IC_{50}, μM)	Cytotoxicity (CC_{50}, μM)
Calanolide A	1.15 (40)	0.425 (40, 58, 59)	0.027 (4)	8.48 (40)
Delavirdine mesilate	0.26 (50, 51)	0.008 (60)	NR	NR
Efavirenz	0.003[a] (52, 53)	2.5 (52, 53)	0.004[b] (70)	NR
MKC-442	0.11 (54)	2.86 (61-64)	0.003 (62)	74.3 (61, 62)
Nevirapine	1.02 (40, 50, 54-56)	0.53 (55, 58, 61, 63, 65-69)	0.27 (66, 67)	233 (61, 65, 66, 71, 72)
Talviraline	0.08 (57)	0.003 (57)	0.002 (57)	NR

All values are expressed as the mean from different experiments using a variety of virus (HIV-1) strains and cell cultures disregarding methods to assess activities. [a]K_i (μM); [b]IC_{90} (μM); NR: not reported. Reference numbers are given in parentheses.

bearing the T1391 amino acid change in reverse transcriptase and exhibits enhanced sensitivity to strains bearing the Y181C amino acid change. In addition, a synergistic response was observed when (+)-calanolide A was combined with the NNRTI, UC781. Additive responses were observed with most NNRTIs and protease inhibitors; no antagonism or synergistic toxicity was observed in these drug combination assays (42).

Anti-HIV activity of (+)-calanolide A was recently reported *in vivo* in a study using the hollow fiber mouse model, in which CEM-SS cells infected with HIV-1 (IIIB strain) were loaded into conditioned polyvinylidene fluoride hollow fibers and implanted (s.c. or i.p.) into SCID mice. (+)-Calanolide A (150 mg/kg/dose b.i.d. or 200 mg/kg/dose once daily by oral gavage for 7 days) significantly inhibited reverse transcriptase activity and increased viable cell mass in the hollow fiber cultures implanted both i.p. and s.c. In animals administered (+)-calanolide A at a dose of 100 mg/kg/dose every 8 h, reductions in reverse transcriptase activity were observed, albeit without protection against cell viability. Viral replication was also inhibited in mice administered (+)-calanolide A i.p. (100 mg/kg b.i.d.). Doses of > 200 mg/kg b.i.d. and 300 mg/kg once daily were found to be toxic. Plasma concentrations of (+)-calanolide A in samples taken on day 7, 24 h after the final dosing, were found to be variable and were usually below the level of quantification. However, a general increase in plasma levels of the agent was observed which correlated with increasing doses and anti-HIV activity (43).

Pharmacokinetics

The pharmacokinetics and bioavailability of (+)-calanolide A (25 mg/kg) were determined after i.v. administration to CD2F1 mice. Using HPLC assays developed for the compound, the AUC, $t_{1/2\beta}$, $t_{1/2\gamma}$ and clearance values were determined to be 9.4 μg/ml/h, 0.25 h, 1.8 h and 2.7 l/h/kg, respectively. Oral bioavailability after administration of 50 mg/kg was 13.2%. No inactive epimer forms of (+)-calanolide A were detected in plasma (44).

Toxicity

(+)-Calanolide A was administered intravenously or orally to rats and dogs for up to 14 or 28 days, respectively. LD_{50} values after i.v. administration were > 20 and > 100 mg/kg, respectively, and > 450 mg/kg after oral administration in both animals. No macroscopic changes or histopathology were noted in rats treated with (+)-calanolide A for 28 days; only gastric hyperplasia was noted at high doses. In contrast, emesis and mucoid feces were observed in dogs treated with high doses for 28 days. Although (+)-calanolide A was negative in mutagenicity tests and was not teratogenic, maternal toxicity was seen in rats with high doses (45).

Clinical Studies

In a phase I study, 47 HIV-negative healthy volunteers were given single doses (200, 400, 600 or 800 mg) or multiple doses (200 mg q.d. or b.i.d. or 400 mg b.i.d.) of (+)-calanolide A for 5 days. A long absorption/distribution phase was observed, with a half-life of approx. 20 h. AUC and C_{max} values were dose-dependent, with levels above the EC_{90} values obtained *in vitro* for several HIV-1 strains. The C_{max} values obtained were higher than expected when considering results from animal studies. In addition, good bioavailability was observed and food had little influence on total drug absorption. The possibility of a gender difference was noted in the single-dose study, although differential effects were not significant in the multiple-dose groups. Adverse events were mild, transient and not dose-related, with the most common including dizziness,

oily taste, headache, nausea, belching and dyspepsia; one grade 3/4 lipase elevation was experienced by a subject receiving the 600-mg single dose (46, 47).

Two phase II dose ranging studies involving HIV-positive patients with no previous antiretroviral therapy are currently under way. Other studies are planned to evaluate the efficacy of (+)-calanolide A in combination with other anti-HIV therapies in HIV-positive patients (46, 48).

Manufacturer

Identified by the Natl. Cancer Inst. (US) and licensed to MediChem Res. Inc. (US), who formed a joint venture with the State of Sarawak, Malaysia, to codevelop the compound under the partership called Sarawak MediChem Pharmaceuticals (48, 49).

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