Podophyllotoxin derivatives: drug discovery and development

Lars Bohlin and Börje Rosén

The exploration and exploitation of podophyllin formulations provide an example of how a plant extract with established ethnomedical use, but also causing toxicity, can provide a basis for new drug discovery and development. Applications and potential applications include the treatment of venereal warts, psoriasis, rheumatoid arthritis, malaria and Alzheimer's disease. This review addresses the history and pharmacological action of these natural products and outlines the preclinical development and clinical trials of drugs in the pipeline and with marketing approval.

Indian podophyllum *Podophyllum emodi* (family Berberidaceae; *emodi* is Latin for the Grecian word for the Himalayas) grows over most of the Himalayan Range[1,2] and is included in *Royle's Illustrated Botany of the Himalayas*, published in 1839 (Ref. 3). The plant has white flowers growing beneath the leaves and large paprika-like fruits; all parts of the plant are highly toxic (Figure 1). The height of the plant varies from 30–90 cm.

Historical applications

The natives of the Himalayan region knew that an aqueous extract of the dried roots of *P. emodi* could be used as a purgative. Extract of the herb was used in China as an antitumour drug more than 2000 years ago[4], and a crude extract was also used in the treatment of snake bites, peridontitis, skin disorders, coughs and against various intestinal worm diseases[5].

Podophyllum peltatum (*peltatum* meaning 'shield-formed'), also known as May apple, mandrake, Indian apple, wild lemon and duck's foot is an indigenous, perennial herb of North America. It has also been used as a purgative and emetic for several hundred years[6]. According to Brunet[7] and Fenton[8] the root was used both as medicine and poison by the native North Americans; it was even used as a suicide agent. John Uri Lloyd stated that podophyllum was used by the Cherokees for deafness and as an anthelminthic agent[9], and the early colonists of the USA learned of the medical properties of the root from the native Indians[10,11].

Medical applications

Podophyllin formulations originated from the rhizome of *P. peltatum*, the extracts of which became so useful in the USA that it was included as a cathartic and cholagogue in the very first *US Pharmacopoeia* published in 1820; in the 1942 edition it was withdrawn. Podophyllum has since been included in other pharmacopoeias, and in 1995 was included in the amended *British Pharmacopoeia*.

Around the turn of last century, podophyllin was tried against diseases such as gout, tuberculosis, gonorrhoea, syphilis, menstrual disorders, dropsy, cough, psoriasis, venereal warts and tumours[12]. Today, the treatment of venereal warts is probably the sole indication.

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Figure 1. Podophyllum emodi *(family Berberidaceae).*

Pharmaceutical preparation

Podophyllin (*Podophyllinum* Lat.) is prepared from an ethyl alcohol extract of *P. emodi* or *P. peltatum*, which is acidified with hydrochloric acid and the sediment cleaned with water. *P. emodi* has mainly been used in Asia, and *P. peltatum* in the USA, Europe and within the British Commonwealth.

The qualitative and quantitative contents of the alcohol/water extracts from the two plants differ considerably. In addition to podophyllotoxin glycosides, the four lignans podophyllotoxin, 4′-demethylpodophyllotoxin, α-peltatin and β-peltatin constitute an essential part of the crude podophyllin extract of *P. peltatum*. In an equivalent extract of *P. emodi* the peltatins are absent.

Resins from the two species differ considerably in podophyllotoxin content; *P. emodi* and *P. peltatum* can contain 40% and 10%, respectively[13]. Picropodophyllin, 4′-demethylpodophyllotoxin and deoxypodophyllin are also present in *Podophyllum* species (Figures 2–5), and the plants also contain flavones, flavonols and flavonoids. Flavonols can be mutagenic and can cause cancer of the bladder. The most well documented flavonol is quercetin, which is mutagenic[14,15]. All parts of the plant – resins, stalks, leaves and fruits – are toxic.

Oral or dermatological poisonings of different podophyllin formulations are highly insidious. On the fourth or fifth day at the earliest, difficulty in breathing and paralysis of the extremities occur. A cumulative dose of only 0.5 mg/kg can be lethal, and if the patient survives, the paralysis can persist for 18 months[16]. Unfortunately, toxicity has been more common in children than in adults, and fetal malformations have been reported following treatment of pregnant women with podophyllin from *P. peltatum*[17,18]. Some 28 deaths have been reported in the literature following oral or dermatological application of podophyllin formulations[16,19].

In 1942, Kaplan[20] first recorded the effectiveness of podophyllin (25% in mineral oil) for the treatment of venereal warts (*Condylomata acuminata*). Since then, other podophyllin in ethanol or benzoic tincture regimens have been developed. After dermatological application, the preparation must always be washed off with water in 2 h. Crystallization out of a solution can render the preparation useless. These highly toxic, mutagenic and unstable formulations remain the principal treatments for venereal warts worldwide.

Because these formulations are crude extracts with low stability, the desired effect cannot be relied upon. In order to ensure effective results from treatment, the solution must be prepared *ex tempore* for each occasion. High toxicity at the treated site is another disadvantage of treatment with the 20–25% solution, irrespective of whether the solution has been newly prepared or whether it is old (more than 2–3 days). This problem is exacerbated by the poor stability, so that it is impossible to know how large a dose of active substance the patient has received. A consequence of this uncertainty has been excessive application in order 'to be on the safe side'. Also, the imported crude podophyllin substance can be infected by fungus.

Thus, the risk associated with treatment with 20–25% podophyllin solution require that applications must be made only by an experienced physician or nurse.

Podophyllotoxin

There are more than 3,000 publications regarding podophyllin, podophyllotoxin and its derivatives. Of these, 65 were

published before 1900. More than 200 natural and semisynthetic podophyllotoxin derivatives are known from the literature.

In 1880, Podwissotzki isolated and established the structural formula of podophyllotoxin from the crude podophyllin mixture[21]. The structural formula was, however, not completely correct. In 1890, Kürsten isolated crystalline podophyllotoxin[22].

Sullivan and coworkers reported in 1948 that the same therapeutic results could be achieved using podophyllotoxin alone rather than the crude podophyllin extract[23]. Around the same time, Hartwell and coworkers isolated α- and β-peltatin from *P. peltatum*, and suggested that these derivatives had a similar effect to that of podophyllin[24]. In 1983, pure crystalline podophyllotoxin was first manufactured on industrial scale by Dr Kurt Leander at Analytecon SA (Couvet, Switzerland).

Podophyllotoxin can also be used to obtain the semisynthetic derivatives etoposide and teniposide (VP 16 and VM 26; Figure 6), which can be shown to possess high cytostatic activity in cell cultures. These compounds were originally developed by Sandoz AG (Basle, Switzerland)[25,26]. They differ chemically only in the substitution of a methyl group (etoposide) for the thenylidene (teniposide) on the glucopyranoside sugar. Both are poorly soluble in water.

Pharmacological activity of podophyllotoxin derivatives

Some podophyllotoxin derivatives bind reversibly to intracellular soluble or solid tubulin. Podophyllotoxin inhibits competitively the binding of colchicine, and binds to tubulin more rapidly than does colchicine[27]. Tubulin, in turn, regulates microtubule assembly.

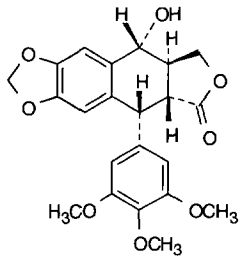


Figure 2. *Podophyllotoxin.*

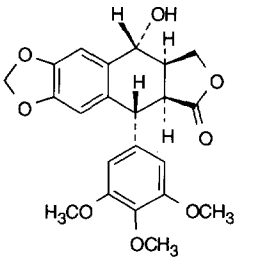


Figure 3. *Picropodophyllin.*

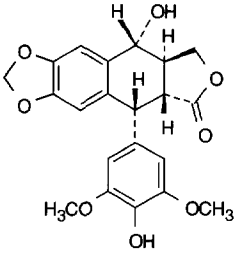


Figure 4. *4'–Demethylpodophyllotoxin.*

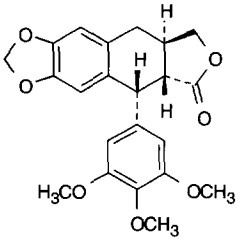


Figure 5. *Deoxypodophyllotoxin.*

Substances with an affinity for tubulin are generally termed 'microtubule inhibitors' because they bind to tubulin, disturbing the equilibrium between microtubule assembly and disassembly.

Microtubule inhibition

The building block of microtubules is tubulin, which is a 6 S dimeric protein of 110,000 M_r. It consists of two nonidentical chains of about 50,000 M_r designated α and β. Polymerization of tubulin dimers produces the protofilaments, which organize to form a microtubule. Microtubules are labile structures; the equilibrium between the microtubule and the pool of free tubulin results from continuous assembly/disassembly at both ends of the microtubule[27–29].

Microtubule inhibitors cause a range of effects, including arrest of the mitotic spindle, arrest of malignant cells invasion and inhibition or acceleration of the release of enzymes and hormones. Microtubule inhibitors may also have a direct effect on the plasma membrane[30]. It has been shown that microtubule inhibitors may prevent the release of chemotactic substances from phagocytic leukocytes in the dot-like attractant assay[31].

Podophyllotoxin concentrations as low as 5 μM cause complete inhibition of tubulin polymerization. In contrast to podophyllotoxin and some other closely related derivatives, neither etoposide nor teniposide affect tubulin assembly at a concentration of 100 μM (Ref. 33). The lower the value of affinity, the higher degree of binding; thus, the relative affinity of picropodophyllin is tenfold weaker than that of podophyllotoxin, and that of benzylidenated podophyllotoxin glucoside is 76-fold weaker (Table 1).

Picro-compounds have a lower biological activity than their respective parent compound.

Highly purified podophyllotoxin and another pharmaceutical entity containing two benzylidenated podophyllotoxin glycosides (CPH 82) were tested clinically in rheumatoid arthritis (Figure 7). Both podophyllotoxin and CPH 82 inhibited mitogen-induced lymphocyte proliferation and immunoglobulin synthesis, and the results helped to determine the optimal dose levels in clinical trials[34].

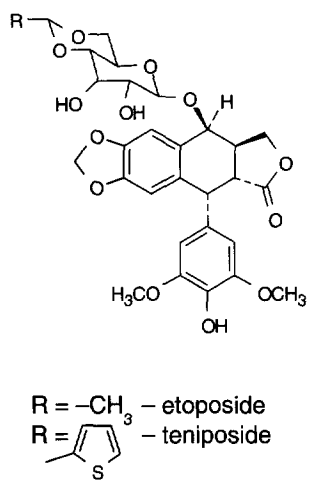


Figure 6. *Etoposide and teniposide.*

Neutrophil chemotaxis

Norberg and coworkers found that podophyllotoxin inhibited neutrophil chemotaxis significantly (by approximately 15%); inhibition was maximal at a podophyllotoxin concentration of 0.01 μg/ml (Ref. 35). Rantapää-Dahlqvist and coworkers chose different CPH 82 concentrations to mimic clinical conditions when they studied the cell cycle *in vitro*. Eight of nine cell lines showed an accumulation of cells in the G_2 phase of the cycle. In several lines a delayed progress through G seemed to occur. Three lines were blocked in both G_1 and G_2. Progression of M-cells seemed only slightly delayed for some cell lines. In comparison with two related 'metaphase' blocking agents, podophyllotoxin, taxol, and CPH 82 exhibited a different and dose-dependent pattern of cell cycle retardation. The authors state 'It is speculated that the cell kinetic action of CPH 82 might give insight into the question why it, unlike other "metaphase" blockers, has proved valuable in the treatment of RA'. The concentrations of CPH 82 were 0.2, 1.0 and 5.0 μg/ml (Ref. 36).

Table 1. Afffinity of podophyllotoxin and derivatives for mouse brain tubulin *in vitro* [27,32]

Substance	M_r	K_i (μM)	Relative affinity
Podophyllotoxin	414	0.51[a]	1
Deoxypodophyllotoxin	397	0.54[a]	1
Podophyllotoxin-β-D-*O*-benzylidene-glucopyranoside (AS 3738)	664	39[a]	76
Etoposide	587	–[b]	–
Teniposide	655	–[b]	–
Picropodophyllin	414	10[a]	20
Colchicine	339	1.1[a]	2

[a]See Ref 27. [b]See Ref. 32

The pharmacodynamics of the epipodophyllotoxins etoposide and teniposide differ completely from those of podophyllotoxin and CPH 82. One reason for this is their interaction with topoisomerase II[37–39].

In contrast to podophyllotoxin, etoposide and teniposide cause an irreversible blockade of cells in the premitotic phase of the cell cycle, leading to an accumulation of cells in the late S and G_2 phases[40]. As discussed above, etoposide and teniposide have no affinity for tubulin and consequently have no effect on microtubule assembly at clinically relevant levels[37,38]. The exact mechanism of action is so far unknown. The cytotoxic effects appear to result from single- and double-strand breaks in DNA and from DNA–protein crosslinks[31,43–45]. However, damage is not observed when the drugs are incubated with purified DNA *in vitro*. Hence, chemical cleavage is not responsible for the effects of etoposide and teniposide on DNA (Ref. 43). Suggested alternative mechanisms include activation of cellular endonucleases, metabolism of epipodophyllotoxins, alteration of the chromatin substrate[46], interaction with topoisomerase II (Ref. 37) and blockade of thymidine formation[38,39].

Anti-inflammatory activity

The epipodophyllotoxins etoposide and teniposide possess no anti-inflammatory properties, they have a high cytostatic activity in cell cultures[47] and are utilized clinically only as anti-tumour agents.

Podophyllotoxin has been investigated for its effect on *in vitro* and *in vivo* immune responses. The following tests were performed in mice using subtoxic doses of podophyllotoxin:

- sheep red blood cell immunization (SRBC),
- skin grafting,
- mitogen stimulation, and
- introduction of mixed lymphocyte reaction and cell-mediated lympholysis.

SRBC test

Using the SRBC test, the first set of experiments were undertaken with intraperitoneal administration of podophyllotoxin once daily, commencing 24 h before injection of the SRBC and continuing until measurement of the splenic B-cell response

in a haemolytic plaque assay. Investigators found that doses of 25 mg/kg inhibited 95% of the response but reduced the spleen cell numbers to 60% of the control values; lower doses, such as 5 mg/kg, suppressed only 35% of the IgM antibody-secreting cells[48]. A second set of experiments showed that lower doses of podophyllotoxin given twice daily were more effective. When podophyllotoxin was given at doses of 2.5 mg/kg or 5.0 mg/kg twice daily, response inhibition rates of 89.0% and 87.2%, respectively, were obtained.

Skin grafts

Using the skin grafts on mice, podophyllotoxin at a dose of 7.5 mg/kg twice daily significantly prolonged the graft survival time when compared to the control animals ($P<0.001$). The median time for treated animals was 15 days compared with 10 days for the controls[48].

Mitogen stimulation

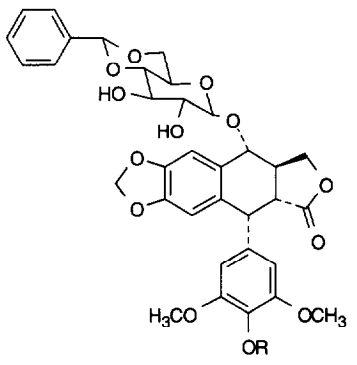
In a test designed to look at mitogen-induced *in vitro* proliferation, podophyllotoxin (5–10 ng/ml) reduced the ^3H thymidine uptake at day 3. Doses of less than 1 ng/ml were found to increase the uptake induced by concanavalin A (ConA) and lipopolysaccharide. Podophyllotoxin was added at the beginning of the culture period. When tested alone, podophyllotoxin did not exert any stimulatory effects. A further experiment in which cells were pre-incubated for 24 h with podophyllotoxin (5 ng/ml) and extensively washed before resuspension in fresh medium established that the mitogen inhibition did not result from direct toxic effects of podophyllotoxin[49].

Cell-mediated lympholysis

Podophyllotoxin inhibited the proliferation response of mouse spleen cells against stimulator cells and the development of specific cytolytic activity. Concentrations of 1.5–3.0 ng/ml caused a very sharp decrease in cell-mediated lympholysis[49]. The drug was apparently very effective when added within 72 h.

Thus, large dose levels of podophyllotoxin have been shown to have an effect on *in vitro* and *in vivo* immune responses. Podophyllotoxin is now obtainable in an extremely pure form. It would be reasonable to suggest that some adverse events observed in the past were largely caused by contaminants in more crude preparations.

CPH 82 is a mixture of two similar benzylidenated podophyllotoxin glucosides (Figure 7). The derivatives are benzylidenated to achieve good intestinal absorption. The mixture provides the best efficient clinical effect. The following anti-inflammatory screening has been performed[50]:



R = H =AS 3739 R = CH_3 = AS 3738

Figure 7. *CPH 82: AS 3739 and AS 3738.*

- UV erythema in the guinea pig,
- granuloma implant in the rat,
- carrageenan-induced paw oedema in the rat, and
- suppression of human lymphocytes *in vitro*.

UV erythema in the guinea pig

Oral dose levels of 0, 5, 20 or 50 mg/kg of CPH 82 were used. The results indicated that at these levels, CPH 82 did not have any significant effect on the erythema induced in the skin of guinea pigs following exposure to UV light.

Granuloma implant and carrageenan-induced paw oedema in the rat

The results of these experiments using oral doses of 0, 5, 20 or 50 mg/kg indicated that CPH 82 did not affect the induction of granuloma tissue or the induced paw swelling at these levels.

The conclusion that may be drawn from the results of the anti-inflammatory studies is that it is unlikely that CPH 82 has classical anti-inflammatory properties.

CPH 82 suppresses lymphocytes in vitro

The objective of this study was to examine whether CPH 82 affects the proliferation and immunoglobulin production response of lymphocytes *in vitro* in response to stimulation from mutagens. Blood samples were taken from normal human donors. The conclusions from the study were that CPH 82 at concentrations achievable in the serum during treatment (>10 ng/ml) have a dose-related suppressive effect on mononuclear cells from blood. This was demonstrated following stimulation with both of the mutagens phytohemagglutinin

(PHA) and ConA; the principal effect was on T lymphocytes. The action of pokeweed mitogen (PWM), which also stimulates B lymphocytes, was suppressed by high concentrations of CPH 82 (>100 ng/ml) (Ref. 51).

Pharmacokinetics

Podophyllotoxin

In a series of tests, two male and two female beagles received podophyllotoxin orally as 1, 2 or 4 soft gelatin capsules per dog, each containing 1.0 mg, and intravenously as a solution of podophyllotoxin at three dose levels corresponding to the oral dose. In the 48-h post-dose period, samples of plasma and urine were obtained for the 6 days between each dose and a rest period of 13 days between oral and intravenous dosing. Although plasma levels generally increased with increasing dose levels, plasma levels following oral doses did not always produce a clear dose–relationship. Peak values were noted 1–4 h post-dose. Initial peak levels following intravenous dosing rapidly diminished and 6 h post-dose were close to zero. Analysis of urine samples did not reveal any clear dose–relationships although, in general, the highest concentration of podophyllotoxin was noted 24–48 h post-dose by either route. Calculation of oral bioavailability ranged from 17.5% for one capsule to 63% for four capsules[50]. Podophyllotoxin passes easily across the blood–brain barrier in dogs.

In an autoradiographic study, ^{3}H-podophyllotoxin was given to pregnant mice either by the oral or intravenous route. Five animals were injected intravenously with the labelled compound at a pregnancy date of 19 days and one at 14 days. The dose of podophyllotoxin used was 1.5 mg/kg in a volume of 0.2 ml. Those animals injected after 19 days of pregnancy were killed at the following times: 5 min, 20 min, 1 h, 4 h and 24 h. The mouse of 14 days' pregnancy was killed 1 h after injection. During the study, the animal that was due to be killed 24 h after injection gave birth. Two of the newborn mice were examined by autoradiography. Three more mice received labelled compound, 0.2 ml orally by cannula. They were examined at 1 h, 4 h and 2h after injection.

Following intravenous injection, the compound passed quickly to the maternal brain and the fetus. Concentrations were higher in fetal organs than in the corresponding maternal organs. Five minutes after administration there were signs of both kidney and biliary excretion. After 24 h, all maternal organs except the liver were cleared. Various levels of accumulation were seen in the bone marrow, spleen, thymus and lymph glands. Low concentrations were seen in the muscles with the exception of the fetal myocardium. At 1 h, the 14-day fetuses showed a somewhat greater accumulation than the 19-day fetuses, but the distribution patterns were similar. At 24 h, the fetal organs were almost completely clear, with activity being noticeable only in the intestinal contents. Those animals receiving the compound by the oral route showed a somewhat different pattern of concentration, although the distribution pattern was similar when compared with the intravenously dosed animals[50]. Maternal liver concentrations were higher and overall concentrations in the fetuses were lower in comparison with the intravenously injected mice. Elimination of radioactivity from the tissues was slower than that observed following intravenous administration.

In pregnant mice, podophyllotoxin penetrates the liver, kidney, spleen and intestine. Podophyllotoxin is mainly eliminated in the faeces[50].

Reproduction studies. Some minor changes were observed in the reproduction studies. High doses of podophyllotoxin had an adverse effect on survival of offspring and delayed the development of survivors. However, podophyllotoxin caused no malformations and had no influence on embryonic or fetal development, fetal weight, fetal size and sex distribution at doses many times higher than the expected clinical dose[50].

Carcinogenicity study. In a two-year rat carcinogenicity study and an 18-month mouse study, the incidence and type of tumour seen in the tissues of animals examined at the end of the studies were similar to those seen in these species. It is therefore suggested that podophyllotoxin has no oncogenic potential.

Etoposide and teniposide

Gastrointestinal absorption of etoposide has been evaluated in adults. The bioavailability is around 50%, but there is a great deal of inter- and intrasubject variability[52,53]. It is, however, possible to increase the systemic bioavailability[54] by an oral intake from drinkable ampoules. Etoposide and teniposide have half-lives of 6 h and 10 h, respectively, and bioavailabilities comparable with those of other neoplastic agents[40]. The mean volume of distribution of steady state is slightly smaller for etoposide than it is for teniposide. Both etoposide and teniposide pass easily across the blood–brain barrier in children, but in adults only low concentrations of etoposide or teniposide have been measurable in the liquor[55]. In mice, teniposide and etoposide have been shown to penetrate into liver, kidney, spleen, brain tissue, heart and intestine[56]. The

degree of protein binding for etoposide is 94.0% compared with 99.4% for teniposide[57].

Approximately 50% of the etoposide and teniposide dose is eliminated in the urine in the first 24 h[58,59]. The dose-limiting toxicity of the epipodophyllotoxins is myelosuppression, mainly leukopenia, occurring 7–10 days after administration[40].

CPH 82 (AS 3738 and AS 3739)

Two benzylidenated podophyllotoxin derivatives, AS 3738 and AS 3739, comprise CPH 82; AS 3738 is methylated in position 4 and AS 3739 is demethylated in the same position (Figure 7).

Because of the limited aqueous solubility of CPH 82, bioavailability studies in the dog cannot be adequately performed. As an alternative, two mass-balance studies were performed in volunteers. Four men and four women received a single dose of AS 3738 and AS 3739. Plasma was collected during 48 h, urine 72 h and faeces 96 h after the oral intake. The recovery was after 96 h[50] (Table 2).

Two studies have been performed on a total of 20 healthy volunteers of either sex. One study was based on a single dose of CPH 82 and the other on multiple dosing over 3 days[50]. Single dosing was undertaken in ten normal volunteers: five men and five women. The average weight and age for the men were 77 kg and 30.6 years, and for the women 63.1 kg and 29.2 years. Each volunteer received a single dose of 50, 100 or 150 mg of CPH 82. An interval of at least 7 days was allowed between each dose.

All haematological parameters measured were within normal limits. Urine and blood values remained within normal limits, and there were no obvious treatment-related trends. The level of absorption of CPH 82 varied significantly between sexes. Urinary excretion was dose-related and was maximal in the first 12 h. Blood pressure and the ECG parameters remained within normal limits throughout the study (Table 3).

The multiple-dosing study was an open pharmacokinetic study performed in ten healthy volunteers (five men and five women). Each volunteer was given 100 mg CPH 82 three times daily for two consecutive days. Dosing took place at 8-h intervals. CPH 82 was given as capsules each containing 50 mg of active ingredient. On days 1 and 2, each subject received 300 mg of CPH 82 and on day 3, 100 mg of CPH 82. Blood samples were taken at intervals throughout the study[50]. Average weights and ages were 78.9 kg and 31.4 years for the men and 61.6 kg and 34.4 years for the women.

Table 2. Average percentage recovery of AS 3738 and AS 3739 from men and women following administration of a single dose

Specimen	AS 3738	AS 3739
Faeces	62.3	57.2
Urine	9.3	4.7
Recovery	71.6	61.9

Biochemical and haematological values were within normal limits for all but one volunteer. The plasma analysis was designed to detect both components of CPH 82 (AS 3738 and AS 3739). The plasma concentrations of AS 3738 at the end of the study on day 3 revealed a slight difference between the sexes. The mean C_{max} was 4,445 ng/ml for men and 3,852 ng/ml for women; the mean t_{max} for men was 1.0 h whereas that for women was 1.5 h. The half-life of AS 3738 was shown to be 4–8 h. In the course of the study, five of the ten volunteers (two men and three women) reported mild adverse events. These generally related to gastrointestinal discomfort, including diarrhoea, loose stool, nausea and flatulence. None of the symptoms was of sufficient severity to cause the volunteer to withdraw.

Carcinogenicity study. A 92-week carcinogenicity study in mice showed that a daily dosing of 50 mg/kg of CPH 82 did not have a tumourigenic effect. In the male top-dose group, the survival rate appeared to be greater than that in the control group. There is no evidence of a tumourigenic effect in mice at dose levels 10–15 times the anticipated clinical dose. In neither animal was target organ toxicity demonstrated.

Reproduction studies. CPH 82 was also found to be without significant effect in different reproduction studies in rats and rabbits using dose levels of up to 200 mg/kg per day. In a dominant lethal study in mice, dose levels of CPH 82 including 400 mg/kg per day were without significant effect on reproductive capacity[50].

Table 3. C_{max} and t_{max} values following single oral doses of CPH 82

Treatment	C_{max} (ng/ml)		t_{max} (h)	
CPH 82 dose	Men	Women	Men	Women
150 mg	2324.9	1428.9	1.00	1.00
100 mg	1765.2	952.5	1.00	1.00
50 mg	596.8	311.9	0.75	0.75

Drug development and therapeutic applications

The ethnomedical use of different podophyllin formulations has resulted in a drug discovery process leading to further development of modern drugs. The epipodophyllotoxins etoposide and teniposide were originally developed by Sandoz and later launched commercially by Bristol-Myers Squibb. They are used successfully worldwide as antitumour agents.

Venereal warts

At local, excessive concentrations of podophyllotoxin, the substance eradicates the infected cell by stopping nucleoside transport across the cell membrane. This biological interference leads to death of the infected cell and, consequently, of the papilloma virus.

Two podophyllotoxin formulations have been developed, an alcoholic solution and a cream, both for topical use. More than 1,300 males and females have been treated in 14 clinical studies with WARTEC® solution or creams (Conpharm; 0.5% podophyllotoxin solution or 0.15–0.30% podophyllotoxin cream). Both formulations have proved to be superior to crude podophyllin formulations with regard to both efficacy and tolerance.

The alcoholic solution is registered in all Western European countries (except Portugal and Austria) and in China, Hong Kong, Singapore, Thailand, the Philippines, Australia, Malaysia, South Africa, Canada and Argentina. The cream formulation of podophyllotoxin has specific advantages, in that it is more convenient to use and makes treatment accessible to more patients.

Psoriasis

Pharmacological investigations have shown that podophyllotoxin decreases cell proliferation at clinical concentrations of podophyllotoxin. Podophyllotoxin also inhibits the release of cytokines such as IL-1 and TNFα (Ref. 50). The clinical efficacy of podophyllotoxin cream has since been confirmed in different clinical studies[50,60,61].

Two studies, one open and one double-blind, have been performed with different concentrations of creams. The purpose of the studies has been to establish effective therapeutic levels, in addition to safety and tolerance. A significant difference between monitored and untreated lesions was observed after only a few weeks of treatment with Psorox® cream (Conpharm), and this difference increased further during the course of the study[50,60].

Malaria

Three clinical studies have been performed in East Africa in which all patients were infected with *Plasmodium falciparum* and were completely resistant to chloroquin. After only 3–7 days' treatment most patients were completely clear[61]. Further studies are planned.

Rheumatoid arthritis

In recent years, some leading rheumatologists have cast doubts over the definition of rheumatoid arthritis as primarily an inflammatory condition. This follows the observation that pain, swelling and immobility are reduced following anti-inflammatory therapy, but tissue destruction and joint damage continue. Observations by Fassbender[62] showed the presence of cell masses involving the synovial tissue. According to the author, the cytological characteristics and aggressive behaviour of these masses were analogous to tumours.

In the past 12 years, studies of the efficacy and safety of two benzylidenated podophyllotoxin glucosides – CPH 82 (Reumacon®; Conpharm) have been performed. More than 500 patients, mostly with severe rheumatoid arthritis, have been documented in open and comparative, double-blind studies[63]. CPH 82 has been shown to be clinically superior to placebo, sulphasalazine, azathioprine and auranofin. Approval of a European marketing application is anticipated in 1997/1998.

Alzheimer's disease

Because of favourable results in the treatment of secondary amyloidosis in connection with rheumatoid arthritis, a double-blind comparative study is ongoing against placebo in the treatment of Alzheimer's disease. A number of types of amyloid are known, depending on the type of protein forming the fibrils. However, all types of amyloid are regarded as pathological; normally occurring amyloid fibrils have never been found. Hence, all amyloid tissues represent abnormal formation of fibrils. It is not yet known why the amyloid fibrils are not completely degraded but are instead incorporated progressively. One explanation may lie in the strong intermolecular bonds in the fibril and possibly also in the protective sheathing of proteoglycans. Amyloid gives rise to a local inflammatory reaction. Alzheimer's disease can be treated with acetyl cholinesterase inhibitors or indomethacin[64].

The pathogenetic importance of the amyloid substance lies, to a large degree, in the space occupied by the substance, with a consequent atrophy of the surrounding tissue. The amyloid, which is deposited between cells and between groups of cells and vessels, probably hinders the transport of nutrients and other substances. The cell membranes may be damaged by penetrating fibrils.

One comparative clinical study against placebo is ongoing, and an evaluation of the results is anticipated in 1997.

Conclusion and future prospects

The aim of this review has been to demonstrate the potential of plants with well-documented ethnomedical use to provide a source for new lead structures that can be used as templates for synthetic modifications, as pharmacological probes and for drug development. However, it also shows that natural products traditionally used for medical purposes can produce serious side-effects and even lethal poisoning. To ensure efficacy and safety, it is necessary to implement a very high level of quality control of the chemical content and bioactivity of a plant extract used as a drug or, preferably, of the isolated active principle.

The studies discussed illustrate the chemical and pharmacological complexity of a plant extract intended for medicinal use and indicate the difficulties, but also the potential, of this type of research. It can be speculated that the search for new bioactive natural products has been too narrowly focused; in-depth studies of a single medical plant extract can reveal multiple pharmacological actions and chemical diversity with new potential applications in drug discovery and development.

REFERENCES

1 Dey, K.L. (1896) in *The Indigenous Drugs of India, 2nd Edition*, pp. 251–254, Thacker Spink & Co.
2 Chopra, R.N. (1933) in *Indigenous of India*, pp. 228–231, The Art Press
3 Royle, J.F. (1839) *Illustrations of the Botany and other Branches of Natural History of the Himalayan Mountains and the Flora of Cashmere*, W.H. Allen & Co.
4 Trease, G.E. and Evans, W.C. (1978) *Pharmacognosy, 11th Edition*, Baillière-Tindall
5 Anon. The Chinese Flora (1972) *Iconographia Cormophytorum Sinicorum, Tomus I*, p. 758
6 Catesby, M. (1731) in *Natural History of Carolina, Florida and Bahama Islands, Tome 1*, p. 24
7 Brunet, O. (1865) in *Catalogue des Plantes Canadiennes Contenues dans l'Herbier de l'Université Laval*, 1 livraison, Québec, pp. 15–16
8 Fenton, W.N. (1941) *Contacts Between Iroquois Herbalism and Colonial Medicine*, Smithsonian Report for 1941, publ. 3670
9 Lloyd, J.U. (1911) *Pharmacy ser. 4, Bull.* 18, 65
10 Bentley, R. (1861) *Pharm. J. and Trans.* 3, 456–464
11 Bennet, A.R. (1903) *Pharm. J.* 70, 238
12 Kelly, M. and Hartwell, J. (1954) *J. Natl. Cancer Inst.* 14 (4), 967–1010
13 Reynolds, J.E.F. (1989) *Martindale: The Extra Pharmacopoeia, 29th Edition*, pp. 928–929, The Pharmaceutical Press
14 Seino, Y. *et al.* (1978) *Mutat. Res.* 58, 225–229
15 Sugimura, T., Nagao, M. and Matsushima, T. (1977) *Proc. Japan Acad.* 53, Ser B, 194–197
16 Filley, C.H. *et al.* (1982) *Neurology* 32, 308–311
17 Cullis, J.E. (1962) *Lancet* ii, 511–512
18 Karol, M.D. *et al.* (1980) *Clin. Toxicol.* 16, 283–286
19 Slater, G.E., Rumack, B.H. and Peterson, R.G. (1978) *Obstet. Gynecol.* 54, 94–96
20 Kaplan, I.W. (1942) *New Orleans Med. Surg. J.* 94, 388–390
21 Podwissotzki, V. (1881) *The Pharmaceutical J. Transactions* 217–219
22 Kürsten, R. (1891) *Arch. Pharm.* 229, 220–248
23 Sullivan, M., Friedman, M. and Hearin, J.T. (1948) *South Med. J.* 41, 336–337
24 Hartwell, J.L. and Shear, M.J. (1947) *J. Cancer Res.* 7, 716
25 Stähelin, H. (1970) *Eur. J. Cancer* 6, 303–311
26 Stähelin, H. (1973) *Eur. J. Cancer* 9, 215–221
27 Kelleher, J. (1977) *Mol. Pharmacol.* 13, 232–241
28 Marveel, M. and De Mets, M. (1984) *Int. Rev. Cytol.* 90, 125–168
29 Margolis, R.L. and Wilson, L. (1981) *Nature* 293, 705–711
30 Edström, A., Erkell, L.J. and Hansson, H-A. (1975) *Virchows Arch. B* 19, 101–113
31 Bäck, O. *et al.* (1978) *Scand. J. Haematol.* 20, 108–116
32 Loike, J.D. (1982) *Cancer Chemother. Pharmacol.* 7, 103–111
33 Loike, J.D. *et al.* (1978) *Cancer Res.* 38, 2688
34 Truedsson, L., Geborek, P. and Sturfelt, G. (1993) *Clin. Exp. Rheumatol.* 11, 179–182
35 Norberg, B. and Bäck, O. (1987) *Curr. Ther. Res.* 41 (2), 173–178
36 Rantapää-Dahlqvist, S. *et al.* (1994) *Br. J. Rheumatol.* 33, 327–331
37 Chen, G.L. *et al.* (1984) *J. Biol. Chem.* 259 (21), 13560–13566
38 Yalowich, J.C. and Goldman, I.D. (1984) *Cancer Res.* 44, 984–989
39 Kalwinsky, D.K. *et al.* (1983) *Cancer Res.* 43, 1592–1597
40 Crom, W. *et al.* (1987) *Clin. Pharmacokinet.* 12, 168–213
41 D'Incalci, M. and Garaffini, S. (1985) *Cancer Chemother.* 7, 98–104
42 Vogelsang, N.J., Raghavan, D. and Kennedy, B.J. (1982) *Am. J. Med.* 72, 136–144
43 Loike, J.D. and Horwizt, S.B. (1976) *Biochemistry* 15, 5443–5448
44 Long, B.N., Musial, S.T. and Brattain, M.G. (1984) *Biochemistry* 23, 1183–1188
45 Wozniak, A.J. *et al.* (1984) *Cancer Res.* 44, 626–632
46 Sinkule, J.A. (1984) *Pharmacotherapy* 4, 61–73
47 Stähelin, H. (1970) *Eur. J. Cancer* 6, 303–311
48 Brigati, C. and Sander, B. (1985) *J. Immunopharmacol.* 7, 285–330
49 Zheng, Q-Y. *et al.* (1987) *Int. J. Immunopharm.* 9 (5), 539–549
50 Conpharm AB, Uppsala, Sweden, internal documentation
51 Truedsson, L., Geborek, P. and Sturfelt, G. (1993) *Clin. Exp. Rheumatol.* 11, 179–182
52 Harvey, V.J. *et al.* (1984) *Proc. Am. Soc. Clin. Oncol.* 30; 24 (90)
53 Smyth, R.D., Pfeffer, M. and Scalzo, A. (1985) *Semin. Oncol.* 12 (Suppl. 2), 48–51
54 D'Incalci, M. *et al.* (1982) *Cancer Chemother. Pharmacol.* 7, 141–145
55 Evans, W.E. *et al.* (1981) *Proc. Am. Assoc. Cancer Res.* 22, 174 (690)
56 Colombo, T. *et al.* (1986) *Eur. J. Cancer Clin. Oncol.* 22, 173–179
57 Allen, L.M. and Creaven, P.J. (1975) *Eur. J. Cancer* 11, 697–707
58 Creaven, P.J. (1982) *Cancer Chemother. Pharmacol.* 7, 133–140
59 Creaven, P.J. and Allen, L.M. (1975) *Clin. Pharmacol. Ther.* 18, 227–233
60 Lassus, A. and Rosén, B. (1986) *Dermatologica* 172, 319–322
61 United States Patent – *Medicinal Uses for Podophyllotoxins.* Patent number 4,788,216
62 Fassbender, H.G. (1986) *EULAR Bulletin* 2, 59–65
63 Larsen, A., Petersson, I. and Svensson, B. (1989) *Br. J. Rheumatol.* 28, 124–127
64 Rogers, J. *et al.* (1993) *Neurology* 43, 1609–1611