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Anti-inflammatory and Analgesic Effects of Drypemolundein A, a Sesquiterpene Lactone from *Drypetes molunduana*

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Abstract

Previous pharmacological screening in our laboratory showed analgesic and anti-inflammatory effects of a crude stem bark extract of *Drypetes molunduana*. Phytochemical studies of this plant led to the isolation an structural elucidation of seven pentacylic triterpenes and one lignan, which were already known compounds, and a new furanosesquiterpene lactone, Drypemolundein A. The purpose of this study was to examine the anti-inflammatory and analgesic activities of drypemolundein A. The compound was studied against carrageenan-induced acute edema. At doses of 10 and 20 mg/kg, orally administered, it significantly reduced (57.57 and 66.66% inhibition at 1 h intervals, respectively) paw edema. At the same doses, this sesquiterpene lactone also exhibited significant analgesic action in force-induced pain in rat paw. These results indicate that drypemolundein A functions as an effective anti-inflammatory and analgesic agent.

Keywords: Analgesic, anti-inflammatory activity, drypemolundein A, *Drypetes molunduana* (Euphorbiaceae).

Introduction

Drypetes molunduana Pax and Hoffm. (Euphorbiaceae) is a woody shrub of the rain forest (Hutchinson & Dalziel, 1958). It grows in the centre, south and eastern provinces of Cameroon where decoctions of some of its parts are used in folk medicine for the treatment of tumors, swelling and inflammation. It is also used as a painkiller. The results of our previous screening on this species showed that the crude extract possessed potent anti-inflammatory and analgesic properties (data not shown). The phytochemical studies we conducted on the same species allowed us to isolate and to elucidate the structure of a number of compounds including sterols, pentacyclic triterpenoids, lignans and a sesquiterpene lactone with unusual structure, named drypemolundein A (Fig. 1) (Wandji et al., 2000). Some of the isolated compounds such as pentacyclic triterpenes are reported to possess interesting anti-inflammatory properties (Safayhi & Sailer, 1997). The novel sesquiterpene lactone, drypemolundein A was found to be the major compound of the extracts of *Drypetes molunduana*. In the study reported in this paper, we examined whether this major compound of the extracts of *Drypetes molunduana* is responsible of the potent anti-inflammatory and analgesic activities exhibited by the crude extracts.

Materials and methods

Plant material

The whole stems of *D. molunduana* were collected at Mintima locality, South Cameroon, in August, 1997. The specimen documenting the collection has been deposited in the National Herbarium, Yaounde (ref. RL5302).

Extraction and isolation

The extraction and isolation procedures have already been detailed (Wandji et al., 2000). In brief, some powdered and

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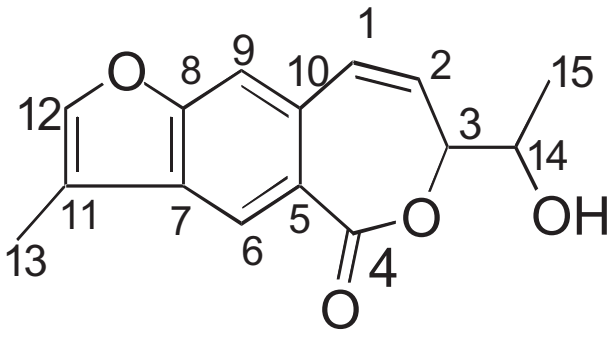

Figure 1. Structure of drypemolundein A.

air-dried stems of *D. molunduana* (1 kg) were extracted with CH_2Cl_2-MeOH (1/1) at room temperature. After evaporation of the solvents under reduced pressure, the crude extract (80 g) was subjected to repeated Si gel column chromatography using hexane, CH_2Cl_2 and MeOH in various proportions. On the basis of TLC, all the resulting fractions were combined in four series A–D. Further column chromatography of series B (6 g), using $CHCl_3$ and EtOAc in increasing proportions yielded drypemolundein A (80 mg). Drypemolundein A was obtained as crystal (CH_2Cl_2), mp 148–150 °C. Its structure was successfully determined using a combination of homo- and heteronuclear two-dimensional NMR techniques (^{1}H-^{1}H COSY, HETCOR, HMQC, HMBC spectra).

Animals

Groups of four male and female Wistar rats weighing 150–200 g were used for anti-inflammatory and analgesic activities. All animals were fed on broilers' marsh with access to water and food *ad libitum*. They were housed in suitable environmental conditions throughout the experiments.

Carrageenan-induced rat paw edema

Edema was induced on the right hind paw of rats by a subplantar injection of 0.05 ml of a solution of 1% sterile carrageenan in saline. The volumes of the injected paw were measured 30, 60, 120, and 240 min after induction of the inflammation using a Plethysmometer (Ugo Basile No 7140). Inflammation was expressed as an increase in paw volume due to a carrageenan injection. Indomethacin (7 mg/kg) and aspirin (20 mg/kg) were used as reference compounds. Drypemolundein A (10 and 20 mg/kg) and the reference compounds were orally administered 30 min before the carrageenan injection. All used drugs were dissolved just before use in 5% DMSO. Control animals received the vehicle only. Edema (ΔV) and inhibition rate (I) were calculated as follows:

$$\Delta V = Vt - Vo$$

$$\%I = 100 - \%\ \text{Inflammation}$$

Where Vt is the right hind paw volume at time t, Vo the hind paw volume before subplantar injection of carrageenan and % Inflammation = ΔV/Vo × 100.

Analgesic tests

The level of pain was measured on rat left hind paws, using an Analgesy-meter (Ugo Basile No 7200), before the administration of the samples dissolved in 5% DMSO; Drypemolundein A (10 and 20 mg/kg), indomethacin (7 mg/kg) and aspirin (10 mg/kg). Pain was induced by the application of a constantly increasing pressure on rat paws. The amount of force that provoked the withdrawal of the rat paw indicated the level at which the pain was felt. Analgesia was measured before and then 1, 2, and 3 h after the oral administration of the samples. Control animals received the vehicle only.

Statistical analysis

The results are expressed as mean ± SEM. Data are analysed statistically by analysis of variance followed by the Student's *t*-test. P values ≤ 0.05 are considered to be significant.

Results

Anti-inflammatory effects of drypemolundein A

The anti-inflammatory effects of drypemolundein A compared with those of indomethacin and aspirin are presented in Table 1. Drypemolundein A administered orally at a dose of 10 mg/kg inhibited edema formation significantly ($p < 0.001$) 30 min after the carrageenan paw injection, that is 1 h after the drug administration; with a percentage of inhibition of 57.57%. At 2 and 4 h, it still has significant anti-edematous effects ($p < 0.05$), but it was less effective than at 1 h. The percentage of inhibition decreased gradually, 40.18 and 21.39%, respectively. When the dose of drypemolundein A was doubled, inhibition of edema was increased to 66.66% at 1 h. The anti-inflammatory activities of this dose, although significantly maintain 2 and 4 h after administration of Drypemolundein A ($p < 0.05$), falls more rapidly than with the dose 10 mg/kg. These data are difficult to interpret. It is more likely that differences in individual pharmacokinetics responsed may account in this type of situation. Drypemolundein A at both doses (10 and 20 mg/kg) seems to be more effective in reducing rat paw edema than indomethacin (7 mg/kg) (57.57 and 66.66% versus 58.33% inhibition) and aspirin (20 mg/kg) (57.57 and 66.66% versus 35.60%) at the 1-h interval. But indomethacin and aspirin are much more powerful in reducing paw edema at 2 h (64.48 and 53.25%, respectively, versus 40.18 and 28.97% inhibition) and 4-h intervals (59.82 and 54.36%, respectively, versus 21.39 and 21.83%).

Table 1. Effects of drypemolundein A, indomethacin and aspirin on acute inflammation induced by carrageenan.

Sample	Dose (mg/kg)	Edema ΔV (ml) ± SEM				%I			
		30 min	60 min	120 min	240 min	30 min	60 min	120 min	240 min
Control	–	1.05 ± 0.16	1.32 ± 0.21	2.14 ± 0.21	2.29 ± 0.20	00.00	00.00	00.00	00.00
Indomethacin	7	0.75 ± 0.12	0.55 ± 0.12**	0.76 ± 0.19**	0.92 ± 0.17*	28.57	58.33	64.48	59.82
Aspirin	20	0.85 ± 0.16	0.85 ± 0.21	1.0 ± 0.28**	1.04 ± 0.31*	19.04	35.60	53.27	54.36
Drypemolundein A	10	0.78 ± 0.07	0.56 ± 0.03**	1.28 ± 0.14*	1.82 ± 0.10*	25.71	57.57	40.18	21.39
	20	0.63 ± 0.11	0.44 ± 0.07**	1.52 ± 0.04*	1.79 ± 0.07	40.00	66.66	28.97	21.83

Edema is expressed as increase in paw volume (ΔV) ± SEM.
n = 4 animals.
* P ≤ 0.05.
** P ≤ 0.001.
%I = Percentage Inhibition.

Table 2. Analgesic of Drypemolundein A, indomethacin and aspirin.

Sample	Dose (mg/kg)	Force (gf) causing pain with time			
		0	1 h	2 h	3 h
Control	–	70.25 ± 10.68	70.00 ± 14.86	70.00 ± 8.89	66.25 ± 2.39
Indomethacin	7	83.75 ± 6.88	71.25 ± 10.87	75.00 ± 5.40	72.50 ± 10.89
Aspirin	20	53.75 ± 10.28	72.50 ± 18.31	65.00 ± 11.90	72.50 ± 6.29
Drypemolundein A	10	61.25 ± 3.14	75.00 ± 6.12	93.75 ± 8.50	105.00 ± 14.71*
	20	73.75 ± 5.54	103.75 ± 8.50*	116.25 ± 5.54**	87.50 ± 3.22

Values are averages of 4 values ± SEM, * P ≤ 0.05, ** P ≤ 0.001.

Analgesic effects of drypemolundein A

The effects of drypemolundein A compared to those of indomethacin and aspirin in reducing mechanical pain, induced by the pusher and plinth of the Analgesy-meter are shown in Table 2. When drypemolundein A was orally administered at the dose of 10 mg/kg, it significantly reduced pain that is mechanically induced in non-inflamed rat paw at the 3-h interval after administration ($p < 0.05$). The effect in reducing the pain sensation was noted at 1- and 2-h intervals, although not yet significant based on Student's *t*-test. The force causing pain was gradually increased from 61.25 ± 3.14 gf before drug administration to 75 ± 6.12, 93.75 ± 8.50 and 105.00 ± 14.71, at 1-, 2- and 4-h intervals, respectively. At the double dose of 20 mg/kg, drypemolundein A showed a more powerful effect. The anti-nociceptive activity was significant at the 1-h interval ($p < 0.05$), and the 2-h interval ($p < 0.001$). The force (gf) causing pain was increased from 73.75 ± 5.54 to 103.75 ± 8.50 and 116.25 ± 5.54 at 1- and 2-h intervals, respectively. At the 3-h interval, this dose remains effective compared to 0 h, but was non-significant when analysed with Student's *t*-test. Again, the observation that the analgesic effect of drypemolundein A should be significant at the 3-h interval with the dose 10 mg/kg but not with the dose 20 mg/kg seems unusual. Whether this should be attributed to specificity in the pharmacokinetics of this drug remains to be elucidated. Indomethacin (7 mg/kg) and aspirin (20 mg/kg) did not show significant anti-nociceptive effects on mechanical paw pain in rats.

Discussion

The carrageenan experimental model of inflammation is highly sensitive to non-steroidal anti-inflammatory drugs, and it has long been accepted as a useful phlogistic tool for investigating new anti-inflammatory drugs. The initial phase of carrageenan paw edema is mediated by histamine and serotonine, while the mediators in the later phase are suspected to be arachidonate metabolites producing an edema dependent on mobilization of neutrophils. It seems that the primary effect of carrageenan as an inflammatory agent is the activation of phospholipase A2 (PLA2), although its cytotoxic effect may initiate further inflammatory actions. Inhibitors of the arachidonate cyclooxygenase (CO) are much more effective than those of the arachidonate lipoxygenase (LO) in inhibiting the carrageenan-induced inflammation (Flower et al., 1985). In relation to the plant extract assayed, edematous response was already very significant 1 h after administration – that is 30 min after the carrageenan

injection (>50% inhibition with both doses). These results suggest that drypemolundein A acts by suppressing the installation of the first phase of the inflammation process. It is thus likely that drypemolundein A acts as the LPA2 inhibitor. One would think that the drug inhibits the cyclooxygenase synthesis, since the anti-inflammatory affects on the carrageenen-induced paw inflammation are very significant; also because the carrageenan inflammatory model basically reflects the action of prostaglandin (Di Rosa & Willoughby, 1971; Flower et al., 1972).

The rapid absorption of drypemolundein A in the gastrointestinal tract may be attributed to the fact that it is a relatively small molecule with no sugars attached. The absence of a free carboxyl group in its structure is another interesting feature; it would not interact with gastric H-receptors, and would not modify gastric acidity. Drypemolundein A is not the first sesquiterpene lactone with anti-inflammatory effects. For example, parthenolide, a sesquiterpene lactone isolated from *Tanacetum parthenium* (Asteraceae) showed anti-inflammatory activities and the induction of cycloxygenase-2 expression has been proven to be the action mechanism (Heptinstall et al., 1992; Hwang et al., 1996).

The second part of the study was concerned with the analgesic ativity of drypemolundein A. Pre-treatment of rats with the extract inhibited pain caused mechanically by a constantly increasing pressure on rat paws by the pusher and plinth of the Analgesy-meter. This system provides a model for the study of non-inflammatory pain. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances, such as prostaglandins, may be minimized in this model. It is therefore more likely that opioid-like analgesic drugs be more effective in inhibiting mechanically-induced pain. Drypemolundein A significantly blocked the pain sensation at both used doses (10 and 20 mg/kg). This compound is then effective against pain in the non-inflamed tissue, and in pain due to sensory nerve stimulation. Either the compound has anaesthetic properties or protects nociceptors or both.

The specificity in the pharmacokinetic properties of this compound may be reflected in the observation that at a dose of 10 mg/kg, the extract seems more effective than the double dose of 20 mg/kg at the 3-h interval. Aspirin and indomethacin did not show analgesic effects on this model of pain. This corroborates previous results that aspirin and aspirin-like drugs are ineffective both against pain in non-inflamed the tissue, and in pain due to sensory nerve stimulation (Flower et al., 1985).

From the same crude plant extract, pentacyclic triterpenes with known structures, erythrodiol, oleanolic acid, hyderagin, and ursolic acid (Mahato & Kundu, 1994), have been isolated. These compounds have also shown to have anti-inflammatory activities (Safayhi & Sailer, 1997). These compounds, with drypemolundein A, may then be acting in synergism. Nevertheless, the eventual importance of the pentacyclic triterpenes as anti-inflammatory agents is likely to be minor, for both their effect and concentration in raw material and extracts are lower than for Drypemolundein A. A new friedelane derivative that has been called Drypemolundein B was also simultaneously isolated from *Drypetes molunduana* (Wandji et al., 2000). We found in our study that this compound at the dose of 10 and 20 mg/kg had no anti-inflammatory, nor analgesic effects on the same animal models (data not shown).

In conclusion, the presented data indicate that oral administration of Drypemolundein A shows anti-inflammatory and analgesic activities. The anti-inflammatory effects appear to be related to an inhibition of PLA2 and arachidonate cyclooxygenase, because the extract seems to be very active in inhibiting the first phase of the inflammatory responses induced by carrageenan. Also, the strong effectiveness of this compound in inhibiting the carrageenan model of inflammation suggests a non-steroidal mechanism of action. Secondary and comprehensive *in vitro* tests, such as anti-PLA2, and anti-COX activities would test this hypothesis. Drypemolundein A has exhibited interesting anti-nociceptive effects on noninflammatory pain, suggesting an opioid-like analgesic property. It is likely that if drypemolundein A acts by blocking prostaglandin synthesis, then it would be very active in the analgesic model of study in which nociceptors are sensitized by prostaglandins such as the Writhings test. Taken altogether, these data indicate that drypemolundein A is a promising molecule with interesting anti-inflammatory and anti-nociceptive properties.

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