A TOXIC COMPOUND FROM THE LEAVES OF *Dyera costulata* Hook. f.

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ABSTRAK

Telah diisolasi suatu senyawa yang bersifat racun dari daun tumbuhan Dyera costulata *Hook. f. berbentuk kristal jarum TL. 260-260 °C yang berdasarkan data spektroskopinya diidentifikasi sebagai asam ursolat (4). Uji toksisitas terhadap anak udang memberikan* LC_{50} *7,9mg/ml.*

INTRODUCTION

In continuation of our study on Sumatran plants, *Dyera costulata* Hook F. (Apocynaceae) locally known as "Jelutung" has been studied. Six indole alkaloids ochrolifuanine A, E and F, 18-dehidroochrolifuanine A, E and F, were isolated from this species collected in Nigeria (Mirland *et al.*, 1989). An antiallergenic compound, 1,3-o-metilmioinositol, was also been found in this plant (Sakurai *et al.*, 1992). Previously we reported isolation of a new bis-indole alkaloid, 18,19-didehydroochrolifuanine from local species of this plant despite the study of configuration of some of its chiral carbons are still in progress (Putri, 1999). Further investigation on this plant revealed that insoluble part of methanolic extract of its leaves in acidic condition was active against Brine Shrimps lethality assay. This paper reports isolation and structure elucidation of the toxic compound from the leaves of this plant.

EXPERIMENTAL

Plant material: *Dyera costulata* Hook.f (2,9 Kg) was collected in logging area of PT Barito Pasifik, Pasir Mayang-Muara Tebo, Jambi Province, in June 1995 (Arbain, *et al.*, 1996). A voucher specimen (DA-RT 5694) was lodged at the Herbarium of Andalas University (ANDA).

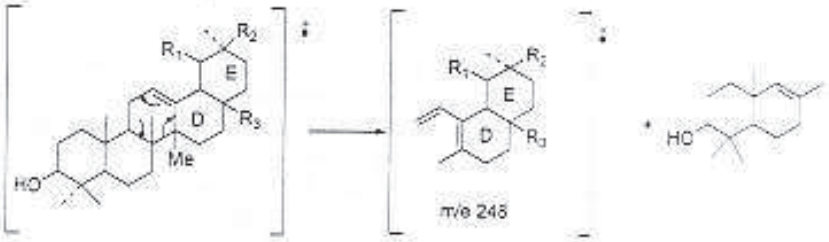
General : Melting point was determined on Fisher Melting Point Apparatus and uncorrected. Electronic spectrum was determined in methanolic solution using Shimadzu UV-VIS 160 spectrophotometer. Infrared spectrum was recorded as potassium bromide disc using a Perkin-Elmer 735B instrument. Proton and carbon NMR spectra were determined in pyridine-d5 solution on a Bruker ARX-500 spectrometer at 500 and 125 MHz for ^{1}H and ^{13}C, respectively and TMS as internal standard. Column chromatography was carried out using silica gel G (Merck, 7743) or sephadex LH-20 (Fluka) as a stationary phase. TLC was performed on silica gel aluminium plates with self indicator at 254 nm (Merck, 5554).

Extraction and Isolation: The air-dried leaves of *Dyera costulata* Hook.f (2.9 kg) were chopped into small pieces and macerated with methanol (10 l) for 3 days. This process was repeated twice more. The methanolic extracts were combined and evaporated under reduced pressure to give a volume ca. 1 l. The extract was acidified with 5% aqueous acetic acid and put aside for overnight. Insoluble material was separated (105 g) and 50 g of this material was triturated with hexane (3 x 500 ml), ethyl acetate (3 x 500 ml) in acid and basic conditions respectively. Ethyl acetate-acid fraction was chromatographed on silica gel with increasing amounts of methanol in ethyl acetate as eluent. Every fraction was subjected to Brine Shrimp lethality assay (Mayer *et al.*, 1982). The active fractions were combined and rechromatographed on Sephadex LH-20 using methanol as eluent to give a compound **(4)** (450 mg) as a needles from methanol-ethyl acetate m.p. 260-262 ^{0}C.

RESULT and DISCUSSION

Eventhough the methanolic extract of this plant was inactive, the insoluble part of this extract in acidic condition showed a significant result on Brine Shrimp lethality assay (LC_{50} 97,8 µg/ml). Ethyl acetate solution of insoluble part in acidic condition showed the highest activity (LC_{50} 34,5 µg/ml). This fraction was chromatographed on silica gel then followed by sephadex LH-20 and yielded compound **(4)** which gave strong toxicity against Brine shrimp LC_{50} 7.9 µg/ml.

The high resolution mass spectrum of this compound gave a molecular ion at *m/z* 456.35 which was in agreement with molecular formula $C_{30}H_{48}O_3$. The electron impact (EI) mass spectrum of (**4**) showed fragment peaks at m/z 441 $[M-CH_3]^+$, 438 $[M-H_2O]^+$ and 411 $[M-CO_2H]^+$. The base peak appeared at m/z 248 due to retro-Diels-Alder fragmentation furnished a very characteristic peak (**3**) for Δ^{12}-oleanenes (**1**) or Δ^{12}-ursenes (**2**) type terpenoids which has a carboxylate group in ring E (Scheme 1) (Djerassi *et al.*, 1962).

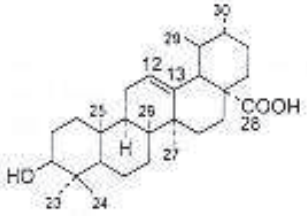


(1) R1 =H, R2=R2 = Me or COOH
(2) R2 =H, R1=R3 = Me or COOH

(3)

Scheme 1

^{1}H-NMR spectra showed a vinyl signal which appeared at δ 5.49 as a multiplet. A triplet at δ 3.46 could be assigned as a proton adjacent to hydroxyl group. The presence of hydroxyl group was confirmed by IR spectrum. ^{13}C NMR spectrum showed that this compound has 30 carbons where with DEPT technique 7 of them are methyl groups, 9 methylenes, 7 methines and 7 quaternary carbons. A carbonyl signal appeared at δ 179.9 which was further confirmed by the presence of C=O stretching at ν 1715 cm^{-1} in IR spectrum. Down field signals appeared at δ 125.6, 139.2 and 78.1 indicated that this compound has an Δ^{12}-ursene skeleton which has a carbonyl and a hydroxyl groups. These allowed us to make a conclusion that this could be ursolic acid. Direct comparison of these data to those of in literature (Kriwacki and Pitner, 1983) supported this assumption.



(4)

Tabel 1. Comparison of ^{1}H (500 MHz) and ^{13}C-NMR (125 MHz) data for 3-hydroxy-12-ursen-28-oic acid (**4**) in pyridine d-5 to those in literature (Kriwacki and Pitner, 1989)

C	^{1}H (**4**)	^{13}C (**4**)	^{1}H	^{13}C
1		39.1		39.1
2		28.1		28.1
3	3.45, 1H, s	78.1	3.46, 1H, s	78.1
4		39.5		39.5
5		55.8		55.8
6		18.8		18.8
7		33.6		33.6
8		40.0		40.0
9		48.1		48.1
10		37.3		37.3
11		23.6		23.6
12	5.48, 1H, m	125.7	5.50, 1H, m	125.7
13		139.3		139.3
14		42.5		42.5
15		28.7		28.7
16		24.9		24.9
17		48.1		48.1
18	2.63, 1H, d	53.6	2.62, 1H, d	53.6
19		39.5		39.5
20		39.4		39.4
21		31.0		31.0
22		37.5		37.5
23	1.24, 3H, s	28.8	1.23, 3H, s	28.8
24	1.01, 3H, s	16.6	1.01, 3H, s	16.6
25	0.87, 3H, s	15.7	0.87, 3H, s	15.7
26	1.04, 3H, s	17.4	1.04, 3H, s	17.4
27	1.22, 3H, s	23.9	1.21, 3H, s	23.9
28	-	179.8	0.98, 3H, s	179.8
29	0.99, 3H, d	17.5	0.99, 3H, s	17.5
30	0.94, 3H, d	21.4	0.93, 3H, s	21.4

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