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The Dichapetalins – Unique Cytotoxic Constituents of the Dichapetalaceae

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1. Introduction

The Dichapetalaceae (syn. Chailletiaceae) is a small family of plants comprising 3 genera and about 165 species. *Dichapetalum* Thouars is the most prominent genus, with 124 species, and is mostly found in the world's tropical and subtropical regions. Irvine lists eight species as occurring in West Africa and Ghana. *D. madagascariensis* (syn. *D. guineense*) and *D. toxicarium* are the commonest and most widely distributed (Irvine, 1961). Hall and Swaine also describe eight species as occurring in Ghana and other parts of West Africa, four of which - *D. barteri*, *D. crassifolium*, *D. filicaule* and *D. heudelotii* - were not mentioned by Irvine. The first two are likely to be new species identified after Irvine. According to Hall and Swaine, *D. heudelotii* includes *D. johnstonii* and *D. kumansiense* which appear on Irvine's list while *D. filicaule* also includes *D. cymulosum* described by Irvine (Hall and Swaine, 1981). Irvine's *D. oblongum* is not mentioned by Hall and Swaine. Several species of the *Dichapetalum* are poisonous to livestock due to the presence of fluorinated compounds, mainly fluorocarboxylic acids (Hall, 1972; O'Hagan *et al.*, 1993). These include *D. toxicarium*, *D. cymosum*, *D. tomentosum* and to a lesser extent *D. barteri*.

D. madagascariensis is one of the less toxic species of the genus. It can grow up to 25m high and about 1.5m in girth. The bark is dry and stringy and peels off in scales. Drooping oval-shaped smooth leaves are arranged alternately in branchlets and grow to about 8 - 16cm long and are 3 - 7cm broad. The numerous tiny flowers occurring in dense heads are yellowish-white in colour and are also fragrant. The orange-yellow ripe fruits are spherical in shape (Irvine, 1961). In the various tropical African communities where it occurs, *D. madagascariensis* finds use in traditional and folk medicine for the treatment of viral hepatitis, jaundice (Lewis and Elvin-Lewis, 1977), sores and urethritis (Burkill, 1985). The fruit pulp and seeds are edible while the plant wood, due to its hardness, is used for domestic purposes (Irvine, 1961).



D. madagascariensis Poir [syn. *D. guineense* (DC.) Keay] A close-up of *D. madagascariensis*, showing the fruit

D. gelonioides (Roxb.) Engler [syn. *Moacurra gelonioides* Roxb.] represents another of the less toxic species and it occurs in wet evergreen forests of South East Asia. In the Philippines, it is reported as treatment for amenorrhea (Fang *et al.* 2006).

Recent separate investigations of *D. madagascariensis* and *D. gelonioides* have led to the isolation and characterization of a novel and unique class of triterpenoids in which a 2-phenylpyrano moiety is annellated to ring A of a dammarane-triterpene skeleton. Biogenetically, their basic structure is characterized by the addition of a C₆-C₂ unit, which might probably be derived from the shikimic acid pathway, to a 13, 30-cyclodammarane-type skeleton (Figure 1). Thirteen dichapetalins, named dichapetalins A – M, have so far been isolated from these two species of the Dichapetalaceae (Achenbach *et al.*, 1995; Addae-Mensah *et al.*, 1996; Fang *et al.* 2006; Osei-Safo *et al.* 2008).

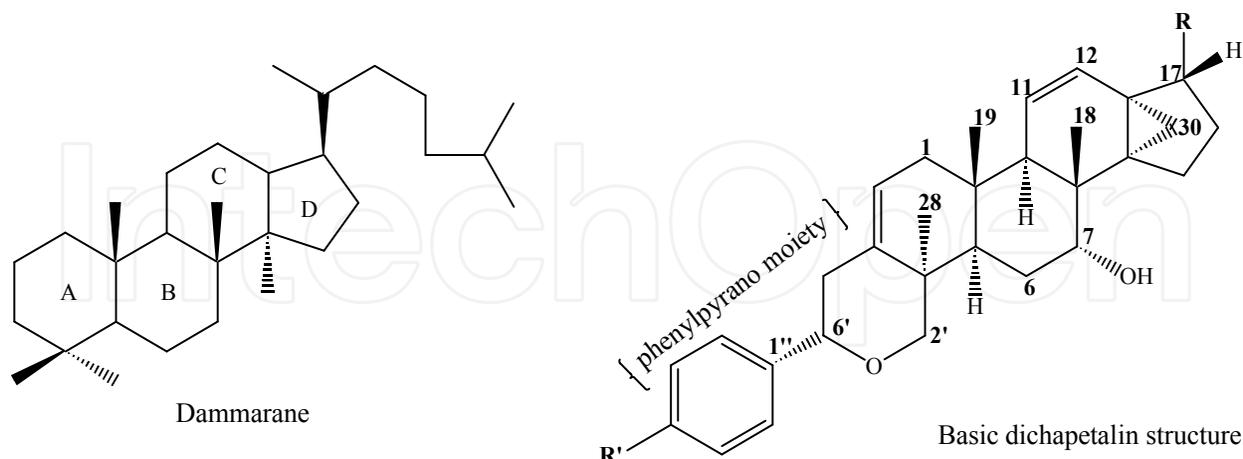


Fig. 1. Structures of dammarane and the dichapetalin skeleton

2. Isolation and characterization

Chromatographic separation of the acetone extract of the whole roots of *D. madagascariensis* collected in Ghana afforded dichapetalin A as the major constituent, together with the minor

components dichapetalins B – H (Achenbach *et al.*, 1995; Addae-Mensah *et al.*, 1996). A separate collection from the same locality gave dichapetalin A and yet another member of the series, dichapetalin M. The absolute configuration of dichapetalin A (Figure 2), the first in the series to be isolated, was determined by single-crystal X-ray diffraction analysis, and has been established to be $4R,5R,7R,8R,9R,10S,13R,14S,17S,20S,23R,6'S$. Its systematic name is therefore [(4 α ,6' α ,7 α ,17 α ,20S,23R,24E)-2',3',5',6'-tetrahydro-7,23,26-trihydroxy-6'-phenyl-13,30-cyclo-29-nordammara-2,11,24-tri-eno[4,3-c] pyran-21-oic acid γ -lactone]. Dichapetalin A is the dextrorotatory isomer, $[\alpha]_D^{21} = +35^\circ$, (Weckert *et al.*, 1996).

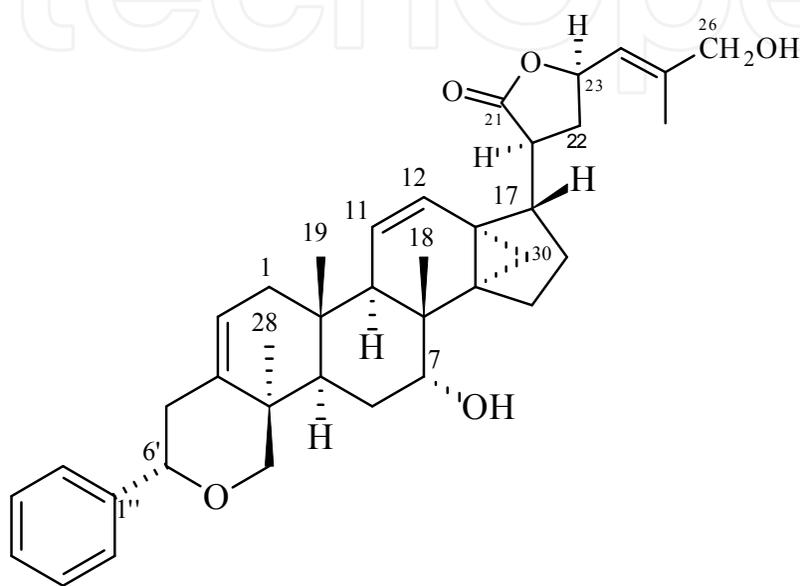
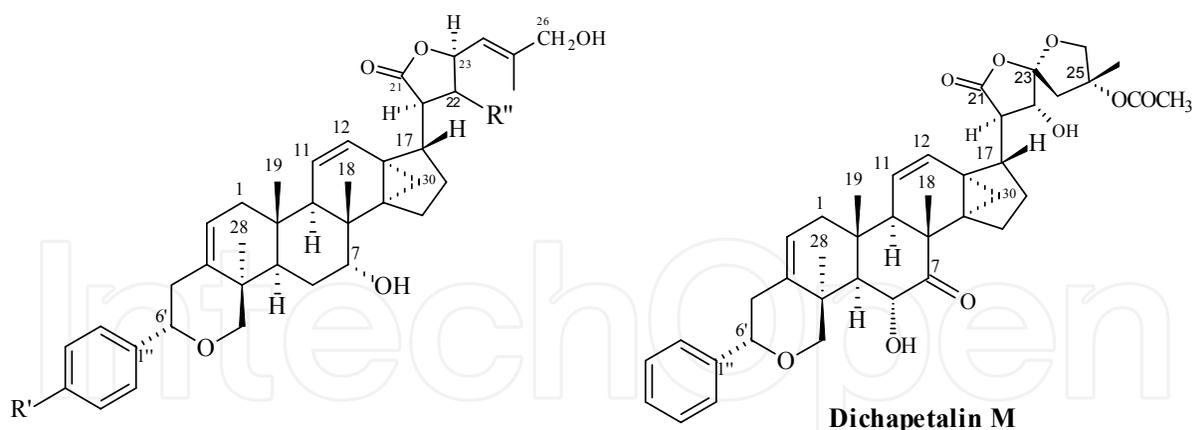


Fig. 2. Dichapetalin A

Meanwhile, between the two investigations of *D. madagascariensis*, Fang and co-workers, on fractionation of the ethyl acetate-soluble extract of the stem bark of *D. gelonioides* (Philippines), isolated dichapetalins A, I and J in one collection and in a re-collection, dichapetalins K and L (Fang *et al.* 2006). The structures of all the compounds were determined on the basis of spectroscopic data interpretation.

So far, only two species of the genus *Dichapetalum* - *D. madagascariensis* (roots) and *D. gelonioides* (stem bark), have produced dichapetalins. The stem bark of *D. madagascariensis* only indicated the presence of dichapetalin A on TLC as part of a complex mixture. The isolated compounds were friedo-oleananes and zeylanol (Darbah, 1994). Zeylanol has been isolated from *D. gelonioides* (Fang *et al.* 2006). Chemical investigation of both the stem and the roots of *D. barteri* did not show any dichapetalins - the isolated compounds were friedelins, including 2 β -hydroxy-3-oxo-D:A-friedooleanan-29-oic-acid, a new triterpene belonging to the friedo-oleanane group, ferulic acid derivatives and the known anticancer lupane triterpenes, betulinic acid and betulonic acid (Addae-Mensah, 2007). *D. gelonioides* also produced betulinic acid (Fang *et al.* 2006).

Basically, the dichapetalins can be distinguished by the nature of the side chain at C-17 which may be grouped into two - the lactone side chain (Figure 3) and the methyl ester side chain (Figure 5). Dichapetalins A, B, I, J, K, L and M possess a lactone side chain while a



Dichapetalin	R'	R''	Other
A	H	H	-
B	H	22 α -OH	-
I	H	H	11,12-dihydro 12- β -OH
J	OMe	H	11,12-dihydro 12- β -OH
K	OMe	H	-
L	H	H	11,12-dihydro

Fig. 3. Dichapetalins with a lactone side chain

methyl ester side chain can be found in dichapetalins C, D, E, F, G and H. Five members of the lactone group, dichapetalins A, I, J, K and L, have an identical side chain comprising a 5-membered lactone with an allyl alcohol substituent. Their structural differences arise from the presence or otherwise of the 11,12-double bond, a 12 β -OH group and a methoxy on the benzene ring of the phenylpyrano moiety. Dichapetalins J and K are methoxylated variants of dichapetalins I and A respectively. According to Fang and co-workers, they are likely to be extraction artifacts due to the initial use of methanol as a solvent. Dichapetalin B on the other hand, is a hydroxylated variant of dichapetalin A.

The uniqueness of the side chain in dichapetalin M is evident in the spiroketal moiety and the C-25 acetoxy group. The oxygenation of C-6 in the basic skeleton is also peculiar to dichapetalin M. A close examination of the side chains of dichapetalins B and M reveals a possible biosynthetic conversion of the former to the latter. Initial hydroxylation and phosphorylation followed by cyclisation could convert the allyl alcohol into a dihydrofuran. Subsequent hydration to the hydroxyl furan followed by acetylation of the hydroxyl by acetyl-CoA could then give the side chain of dichapetalin M (Figure 4).

The methyl ester group consists on one hand, of an open chain terminating in a primary alcohol (dichapetalins C and F) or its stearic acid esterified analogue (dichapetalin D). On the other hand, the primary alcohol cyclizes with the oxo substituent at C-23, to give either a 3-methylfuran moiety (dichapetalin E) or a cyclic methyl ketal (dichapetalins G and H). Dichapetalins G and H are isomeric methyl ketals with the 11,12-dihydro basic skeleton (Figure 5).

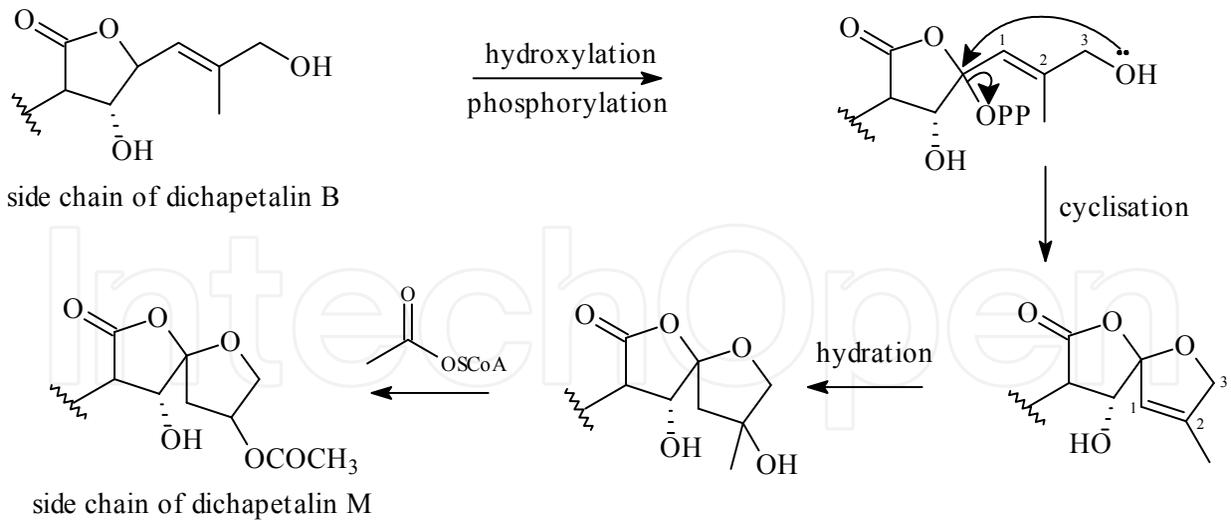


Fig. 4. Proposed biosynthetic pathway for the side chain of dichapetalin M

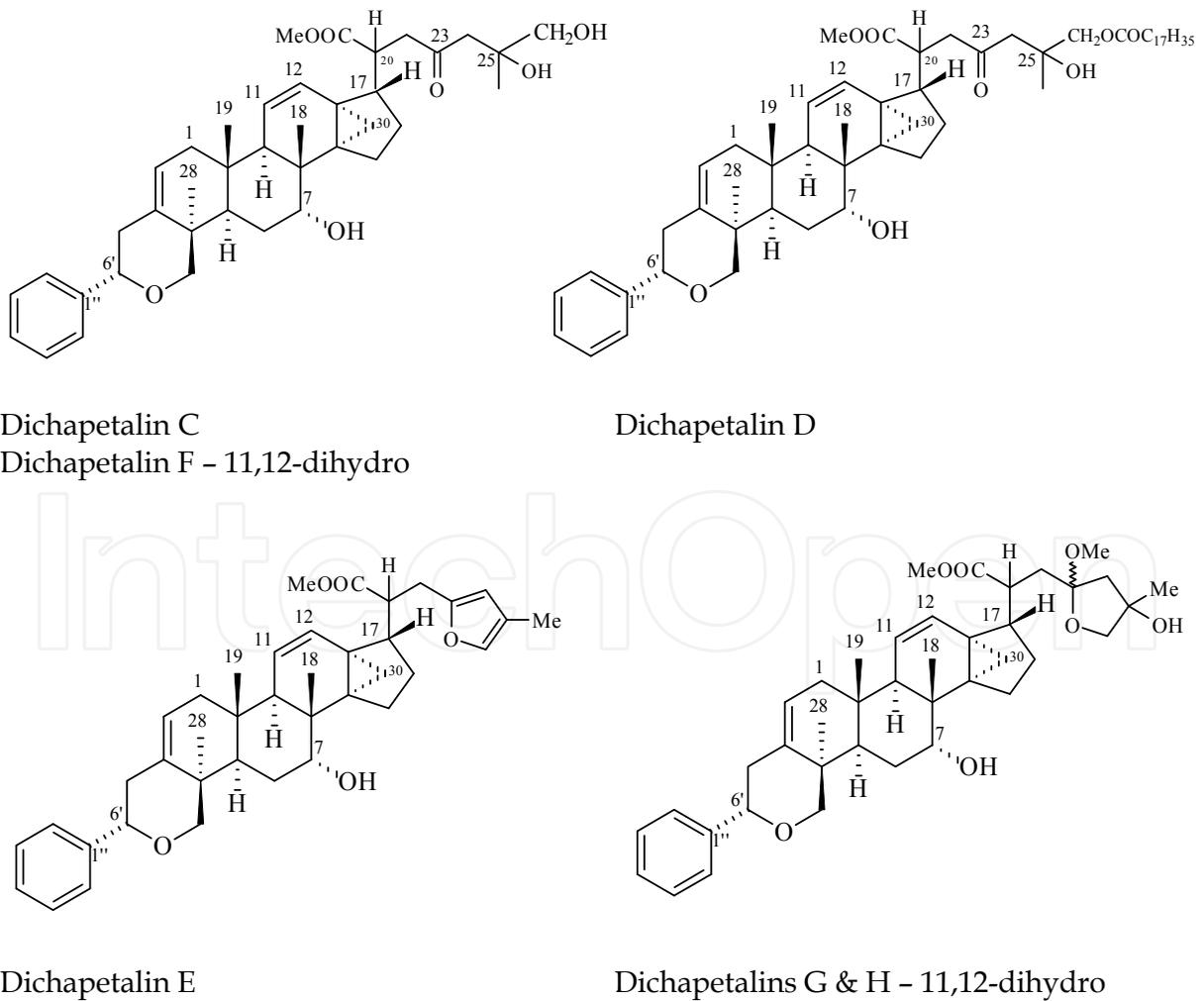


Fig. 5. Dichapetalins with a methyl ester side chain

3. Biological activity

Biological assays including brine shrimp and anticancer studies have so far shown significant activity only with dichapetalins possessing a lactone side chain. None of the methyl ester side chain dichapetalins exhibited significant cytotoxicity.

3.1 Brine shrimp test

The dichapetalins were assayed in the Brine Shrimp Lethality Test according to established protocols (Meyer *et al.*, 1982; Anderson *et al.*, 1991). Dichapetalin A exhibited pronounced cytotoxicity ($LC_{50} = 0.31 \mu\text{g/ml}$), exceeding that of podophyllotoxin by 7-fold while dichapetalin M was 28-fold ($LC_{50} = 0.011 \mu\text{g/ml}$) more potent than dichapetalin A. Dichapetalin C was active to a lesser extent while dichapetalins D & F were almost inactive.

3.2 Antitumour studies

Dichapetalin A showed significant inhibition to cell growth in various cancer cell systems *in vitro*. The sensitivity of the respective systems was however, highly different. L1210 murine leukaemia cells were extremely sensitive ($EC_{90} < 0.0001 \mu\text{g/ml}$) while human KB carcinoma and murine bone marrow stimulated with GM-CSF were affected by concentrations four orders of magnitude higher. *In vivo* tests were also not encouraging (Achenbach *et al.*, 1995; Addae-Mensah *et al.*, 1996). Fang and co-workers further demonstrated selective and significant cytotoxicity in dichapetalins A, I and J ($IC_{50} = 0.2 - 0.5 \mu\text{g/mL}$) against the SW626 human ovarian cancer cell line. Dichapetalins K and L showed broader cytotoxicity against the same cell line. Their study confirmed the loss of activity of dichapetalin A when evaluated in the *in vivo* hollow fiber model (Fang *et al.* 2006). Loss of activity of dichapetalin A *in vivo* is likely to be due to enzymatic hydrolysis of the lactone to an open chain carboxylic acid (methyl ester side chain). Dichapetalin M is yet to be evaluated for its anti tumour potential which, based on its extremely high toxicity towards the brine shrimp, is expected to be more potent than that of dichapetalin A. Moreover, it is envisaged that the unique spiroketal bicyclic side chain of dichapetalin M will confer stability on the lactone, reducing the possibility of ring opening and hence, likely to result in retention of activity *in vivo*.

3.3 Anti-HIV assay

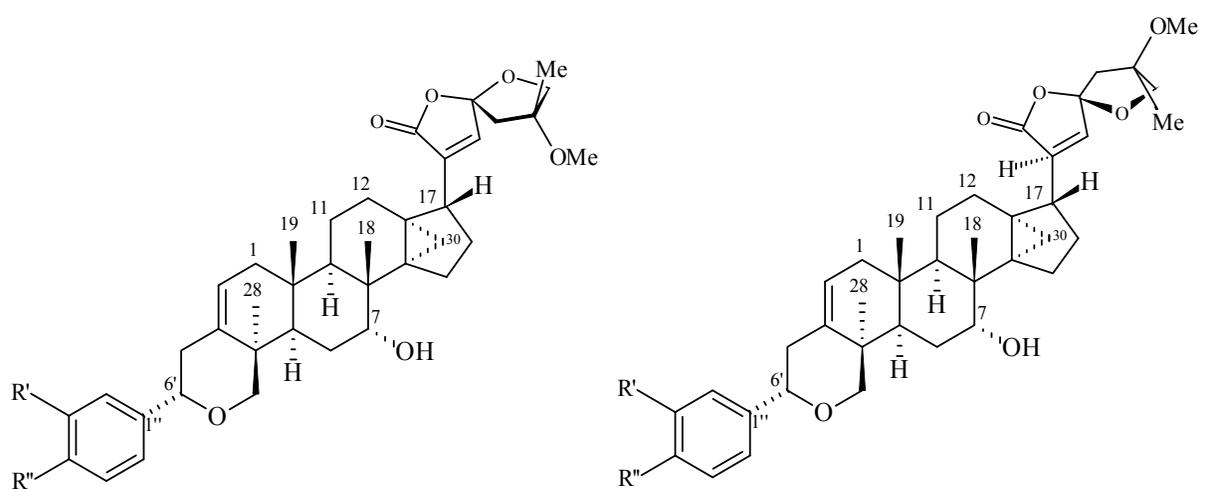
The Tetrazolium-based colorimetric selective assay was employed in the anti-HIV activity test of dichapetalins A and M against HIV-1/IIIB in MT-4 cells as previously published (Ayisi *et al.*, 1991). They both elicited activity at concentrations that were toxic to the cells and therefore did not exhibit any appreciable selectivity in its anti-HIV activity. The aqueous extract of the plant, however, gave an antiviral index of 4.7, an indication of the presence of some level of anti-HIV principle. This may be an indication that the plant contains other compounds that may either be anti-HIV on their own, or in combination with the dichapetalins.

4. Dichapetalins from other sources

The isolation of five new dichapetalin-type triterpenoids, acutissimatriterpenes A – E, from the aerial parts of *Phyllanthus acutissima* (Euphorbiaceae) by Tuchinda and co-workers

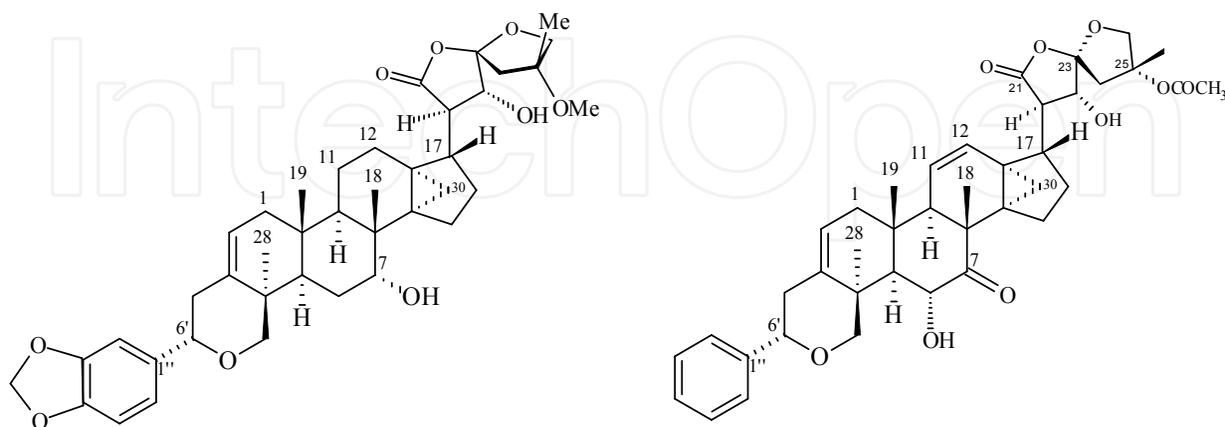
(Tuchinda P. *et al*, 2008) has been reported (Figure 6). This finding of the dichapetalins from a different plant family is of significant taxonomic importance.

Like the dichapetalins, the C-17 side chain is a distinguishing structural feature among the acutissimatriterpenes. Another structural difference is the presence or otherwise of a methylenedioxy unit in the phenylpyrano moiety. This structural unit is peculiar to the acutissimatriterpenes – it has so far not occurred in the dichapetalins. Acutissimatriterpenes A and C are methylenedioxy analogues of acutissimatriterpenes B and D respectively.



Acutissimatriterpene A: $R', R'' = \text{O-CH}_2\text{-O}$
Acutissimatriterpene B: $R', R'' = \text{H}$

Acutissimatriterpene C: $R', R'' = \text{O-CH}_2\text{-O}$
Acutissimatriterpene D: $R', R'' = \text{H}$



Acutissimatriterpene E

Dichapetalin M

Fig. 6. Acutissimatriterpenes A - E

Based on the side chain alone, acutissimatriferpenes A and B are isomeric ketals of acutissimatriferpenes C and D. In terms of the tetrahydrofuran configuration, acutissimatriferpenes A, B and E bear similar side chains – the difference is the hydroxylated alkene in the latter. It is interesting to note the resemblance of their spiroketal side chain to that of dichapetalin M where the acetoxy group at C-25 in the latter has been replaced with a methoxy substituent. Thus, the acutissimatriferpenes can be categorized into the lactone side chain group of the dichapetalins.

As mentioned earlier, structure-activity relationship (SAR) studies revealed significant cytotoxic activity with dichapetalins in the lactone side chain group. Results of biological testing of the acutissimatriferpenes for cytotoxic effects against a panel of six cancer cell lines (P-388 murine lymphocytic leukaemia, human KB nasopharyngeal carcinoma, MCF-7 human breast cancer, Lu-1 human lung cancer, Col-2 human colon cancer and ASK rat glioma) is reported (Tuchinda P. *et al*, 2008). Acutissimatriferpene E exhibited significant activities against P-388 murine lymphocytic leukaemia ($EC_{50} = 0.005\mu\text{g/ml}$), MCF-7 human breast cancer ($EC_{50} = 1.1\mu\text{g/ml}$) and Lu-1 human lung cancer ($EC_{50} = 3.1\mu\text{g/ml}$). Acutissimatriferpenes A and B gave $EC_{50} = 0.4$ and $0.5\mu\text{g/ml}$ respectively against P-388 murine lymphocytic leukaemia. The remaining cell lines did not show activity. Insignificant activities ($EC_{50} > 5\mu\text{g/ml}$) were reported for acutissimatriferpenes C and D against all the cell lines tested. A comparison of these results with those obtained from cytotoxicity test of the dichapetalins indicate that dichapetalin A was ten-fold more sensitive ($EC_{90} < 0.0001\mu\text{g/ml}$) than acutissimatriferpene E against a different strain of murine leukaemia cells. Human KB nasopharyngeal carcinoma exhibited low sensitivity against all the acutissimatriferpenes ($EC_{50} > 5\mu\text{g/ml}$) whereas in a different study, human KB squamous carcinoma was affected by dichapetalin A at $EC_{90} = 1.8\mu\text{g/ml}$.

SAR consideration of the acutissimatriferpenes suggests that the methylenedioxy moiety may not be required for activity but rather, the tetrahydrofuran configuration in the side chain of acutissimatriferpenes A, B and E. All three compounds gave encouraging cytotoxicity against some of the cell lines tested. Both acutissimatriferpenes C and D possess identical side chains, with a tetrahydrofuran configuration opposite that of acutissimatriferpenes A, B and E. Acutissimatriferpene C is the methylenedioxy variant of acutissimatriferpene D, and both did not exhibit appreciable cytotoxic activity against any of the cancer cells. Possibly, the presence of the hydroxylated alkene found in acutissimatriferpene E alone, also enhances cytotoxicity. These deductions confirm the earlier predicted activity of dichapetalin M. It seems to possess all the appropriate structural features for activity – the hydroxylated alkene, the required orientation of the spiro-lactone and the absence of the methylenedioxy unit. The effect of the acetoxy substituent on activity, however, remains to be tested.

Anti-HIV-1 activity employing cell-based assays against MC99 virus and 1A2 cell line system showed various levels of activity with acutissimatriferpenes A – E (Selectivity index = >1.5 - >8.1). In an HIV-1 RT assay, acutissimatriferpenes A and B were moderately sensitive (> 50 to 70% inhibition at $200\mu\text{g/ml}$) followed by acutissimatriferpenes D and C at 37% and 11% inhibition respectively. Acutissimatriferpene E was the least active (-0.5% inhibition). Both dichapetalins A and M also failed to exhibit any appreciable selectivity in anti-HIV activity against HIV-1/IIIB in MT-4 cells.

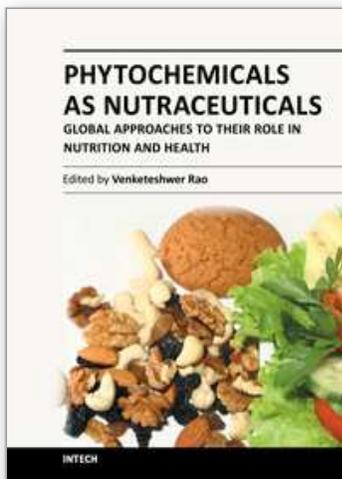
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