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DYSOBININ, A NEW TETRANORTRITERPENE FROM *DYSOXYLUM BINECTARIFERUM**

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Key Word Index—*Dysoxylum binectariferum*; Meliaceae; CNS depressant; tetranortriterpene; meliacin; dysobinin; dysobindiol.

Abstract—Dysobinin, a new tetranortriterpene of the meliacin group showing general CNS-depressant action and mild anti-inflammatory activity was isolated from the fruits of *Dysoxylum binectariferum*. Alkaline hydrolysis of dysobinin gave a new diol named dysobindiol. The structural elucidation of dysobinin and dysobindiol is described.

In our project of screening Indian plants over a wide range of biological activities, we found that a 50% aq. EtOH extract of *Dysoxylum binectariferum* (Meliaceae) [1] showed CNS depressant activity [2]. From the alcoholic extract of air dried powered fruits a crystalline active principle named dysobinin (**1**), mp 185–7°, $[\alpha]_D^{CHCl_3}$ + 150° was isolated in about 2% yield. Structural elucidation by the usual physico-chemical techniques established it to be a new tetranortriterpene belonging to the meliacin class of compounds. Dysobinin showed significant general CNS-depressant action and mild anti-inflammatory activity.

Dysobinin (**1**) analysed for $C_{30}H_{38}O_6$ (M^+ 494) and showed UV absorption at λ_{max}^{EtOH} 237 nm, indicating the presence of an α,β-unsaturated carbonyl chromophore in the molecule. This was supported by the presence of carbonyl bands at 1660 cm^{-1} (cyclohexenone) and an acetyl carbonyl absorption at 1725 and 1260 cm^{-1} in the IR spectrum of **1**.

Dysobinin on mild alkaline hydrolysis yielded a new diol, dysobindiol (**2**) $C_{26}H_{34}O_4$ (M^+ 410), mp 209–10°, $[\alpha]_D^{CHCl_3}$ + 72.2°. The IR spectrum of **2** showed the presence of a hydroxyl function (3550 cm^{-1}), an α,β-unsaturated carbonyl (1650 cm^{-1}); the absorption due to acetyl carbonyl was absent.

The NMR spectra of **1** and **2** revealed the presence of a β-substituted furan ring at C-17, a common feature of meliacins, showing signals at δ 6.23 ppm (β-H) and 7.2 and 7.33 ppm (two α-H). The other notable feature in the NMR of **1** and **2** was the presence of a pair of doublets centred at 5.85 and 7.10 ppm (J = 10.0 Hz) due to two olefinic protons of the disubstituted double bond. The five angular methyl groups resonated between δ 0.8–1.33 ppm, indicating that no oxygen substituents were present on any of them. The two acetoxy methyl protons appeared as a singlet at δ 1.97 (6H) ppm in the NMR of **1** which was absent in that of **2** and instead the latter showed two exchangeable protons on D_2O shake. In the olefinic region, a three proton multiplet centred at δ 5.4 ppm in dysobinin collapsed to a one proton diffused triplet at 5.6 ppm (J = 2.0 Hz) in dysobindiol, which could be assigned to a trisubstituted double bond. The other two protons of the three proton multiplet in **1** were assigned to protons *alpha* to the two acetoxy groups which were shifted on deacetylation and appeared as a broad multiplet centred at δ 4.05 ppm (J = 3.0 and 4.0 Hz) in **2**. These two carbinolic protons on addition of TAI shifted to 5.60 ppm, overlapping the olefinic protons as in **1** and the two carbamate N-H protons in turn appeared at 8.33 and 8.76 ppm as singlets. The corresponding shifts [3] of carbinolic protons by > 1 ppm on addition of TAI indicated that both the hydroxyl groups in **2** were secondary in nature.

The CD curve of dysobinin showed a negative Cotton effect $(\theta)_{345} = -0.72 \times 10^{-2}$, while that of dihydrodysobinin (**3**) obtained by catalytic hydrogenation of **1**, had a + ve Cotton effect $(\theta)_{296} = +0.49 \times 10^{-2}$, and was in agreement with Δ^1-3-keto-4,4,8-trimethyl steroids [4] having rings A and B *trans* fused. Thus the proton at C-5 in **1** and **2** was assigned as *alpha* axial.

The presence of the 3-keto group in dysobinin was also evident from the MS of the ketal derivative (**4**) of dihydrodysobinin (**3**) showing the presence of a base peak at *m/e* 99 corresponding to ion *a*.

The presence of the two hydroxyl groups vicinal to each other in **2** was supported by the periodate oxidation. Dysobindiol consumed one mole of sodium periodate and yielded a new dialdehyde (**5**), $C_{26}H_{32}O_4$, mp 132–3°. The NMR spectrum of **5**, amongst other peaks showed the presence of two aldehydic protons at δ 9.4 (*s*, 1H) and 10.05 (*d*, J = 4 Hz) ppm, the latter being coupled to either the C-5 or the C-9 proton. This limited the placement of the hydroxyl groups either at C-6, C-7 in ring-B or at C-11, C-12 in ring-C, as in both these cases one of the aldehydes obtained would be secondary in nature and the other tertiary. The placement of hydroxyls at C-6, C-7 was confirmed by the MS of **5** which gave fragments *b* and *c* that could arise only by the cleavage of the C-9:C-10 bond. The C-5 proton in **5** was

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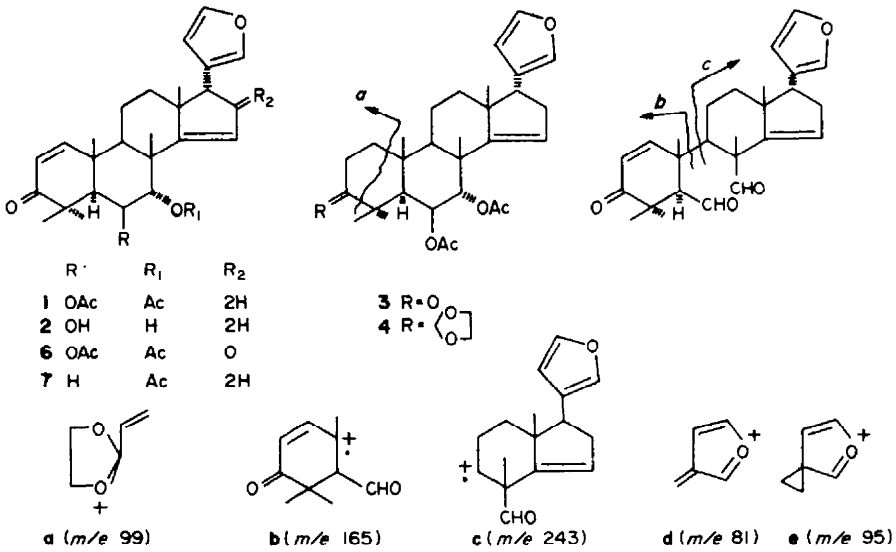

deshielded and appeared as a doublet at δ 2.7 (J = 4 Hz) ppm, coupled with the newly generated aldehyde proton.

Dysobindiol failed to yield an isopropylidine derivative, indicating that the two hydroxyl groups were *trans* to each other. Further, there was no evidence of axial, axial couplings of the C-5, C-6 and C-7 protons in the NMR spectra of **1** and **2** and the hydroxyls at C-6 and C-7 could possibly have the β-axial and α-axial configurations respectively. Dysobindiol on reacetylation (Ac_2O–Py) yielded dysobinin, indicating that no change in configuration took place during alkaline hydrolysis of dysobinin to dysobindiol.

The mass spectra of **1**, **2** and **3** gave rise to a fragment *d* (*m/e* 81) due to a β-substituted furan ring at C-17 and another peak at *m/e* 95 due to fragment *e* which could arise by the cleavage of C-15:C-16 bond facilitated by the presence of a C-14 double bond. This also confirmed the absence of a carbonyl group in ring-D and was in agreement with the cracking pattern of other meliacins [5,6]. The presence of the trisubstituted double bond at C-14 was confirmed by SeO_2 oxidation of **1** when a new diketone (**6**), $C_{30}H_{36}O_7$ mp > 300° was obtained, which had a carbonyl absorption at 1710 cm^{-1} for cyclopentenone [6].

Dysobinin is an example of the growing family of meliacins [5–7] and is of chemotaxonomic importance as it is a 6-acetoxy derivative of azadirone (**7**) occurring in *Melia azadirachta* [6] a plant of the same family.

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STEROLS OF *ALTERNARIA KIKUCHIANA*

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Although sterols have been isolated from many fungi, there are only two reports of their identification in *Alternaria* species. Aizina and Zlatoust [1] reported that ergosterol was the main sterol of *A. brassicicola* and *A. tenuis* and Brown and Jacobs [2] obtained ergosterol peroxide from *A. dianthicola*. An unidentified steroid, mp 180°, was isolated by Sugiyama *et al.* [3] from culture filtrates of the phytopathogenic fungus *A. kikuchiana*.