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NIVENOLIDE, A DITERPENE LACTONE FROM *CROTON NIVEUS**

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Key Word Index—*Croton niveus*; Euphorbiaceae; nivenolide; labdane diterpene.

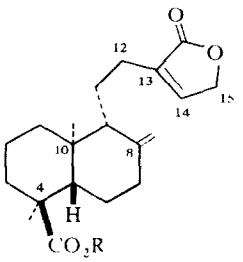
Croton niveus, Jacq, (copalchi) has been used as a popular medicine in Curacao [1]. In a previous study of this species, quercetin was isolated [2].

We undertook the study of this species collected in the region of Tuxtlas, Veracruz. Purification of the $CHCl_3$ soluble fraction of the EtOH extract of the leaves permitted us to isolate a new diterpene lactone, nivenolide, **1a**, $C_{20}H_{28}O_4$, mp 170–171°; $[\alpha]_D$ −38.46 (3.25, $CHCl_3$). Its IR spectrum showed the presence of a carboxylic acid (3300 and 1695 cm^{-1}), a γ lactone function (unsplit absorption at 1750 cm^{-1}) and an exocyclic methylene group (1645 and 900 cm^{-1}). The PMR spectrum showed two singlets at 0.73 and 1.17 ppm which correspond to Me groups on fully substituted carbon atoms. The presence of the exocyclic methylene group was confirmed by the absorption at 4.63 and 4.9 ppm (*br s*, 1H each). A signal observed at 4.77 (*q*, J = 2 Hz, 2H) was assigned to the C_{15} methylene group. The vinylic proton at C_{14} appeared as a quintet at 7.10 (1H).

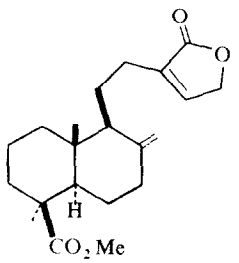
Treatment of nivenolide **1a**, with CH_2N_2–Et_2OH gave the Me ester **1b**, mp 150–151°, which showed in the PMR spectrum the Me ester absorption at 3.68 ppm. The signal at 4.77 ppm attributed to C_{15} methylene group, appeared as a very clear quartet, double irradiation experiments allowed us to show that the C_{15} protons are homoallylically coupled to the protons at C_{12}.

All the spectroscopic data observed for nivenolide methyl ester **1b** (see Experimental) show a great resemblance to the data described for pinusolide [3]**2**. The difference in mps (pinusolide mp 82–83°, nivenolide Me ester mp 150–151°) and specific rotation (pinusolide $[\alpha]_D$ +64°, nivenolide $[\alpha]_D$ −38.46) indicated a difference in one or more assymetric centers. The chemical shifts of the C-4 Me group (1.17 ppm) and C-10 Me group (0.7 ppm) suggested an eperuane squeleton [4] and indicated that the C-4 carboxylic group is equatorially oriented in nivenolide.

These stereochemical deductions were confirmed when the keto dicarboxylic acid **3** was obtained on ozonolysis of nivenolide followed by alkaline H_2O_2 treatment. The product **3** showed mp 208–212°, $[\alpha]_D$ +22.43 (*c* 2.33) identical to the data described for the same compound obtained from polyalthic acid [5].



1a (R = H)
1b (R = Me)



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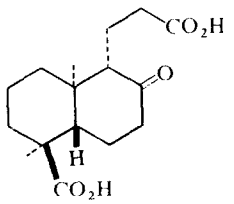

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EXPERIMENTAL

Mps are uncorr. IR spectra were recorded in $CHCl_3$, UV in 95% EtOH and PMR spectra in $CDCl_3$ unless otherwise stated. Analysis were determined by Dr. F. Pascher, Bonn, Germany.

Isolation of nivenolide. Leaves of *C. niveus* (2.5 kg) dried and ground, were extracted twice with EtOH (6 l. each). The EtOH extract was concd to 25% of its vol., and H_2O was added. The aq. EtOH soln obtained after filtration, was extracted with $CHCl_3$. The $CHCl_3$ soln was concd to yield 72 g of crude extract, which was chromatographed over Si gel (1 kg). The fractions eluted with C_6H_6–EtOAc (9 1) yielded the nivenolide, **1a**, (3 g). The analytical sample was prepared from Me_2CO–isopropyl ether and showed mp 170–171°, $[\alpha]_D$ −38.46 (*c* 3.25), ν_{max} 3350, 1750, 1645, 900 cm^{-1}, δ 0.73 (*s*, C-10-Me), 1.17 (*s*, C-4-Me), 4.63 and 4.9 (*br s*, 1 H each CH_2=-8C), 4.77 (*q*, H-15, J = 2 Hz, 2H), 7.1 (*q*, H-14, 1H). (Found: C, 72.0; H, 8.49; O, 19.54. $C_{20}H_{28}O_4$, requires. C, 72.26, H, 8.49; O, 19.25%). The Me ester, **1b**, was prepared in the usual manner with CH_2N_2–Et_2O. The analytical sample prepared from Me_2CO–hexane, showed mp 150–151°, λ_{max} 214 nm (ε, 6330), ν_{max} 1775, 1720, 1645, 900 cm^{-1}; δ 0.73 (*s*, C-10-Me), 1.17 (*s*, C-4-Me), 3.68 (*s*, COOMe), 4.65 and 4.89 (*br, s*, CH_2=C_8), 4.77 (*q*, J = 2 Hz, H-15, 2H), 7.11 (*q*, H-14, 1H) (Found. C, 72.65; H, 8.86, O, 18.64. $C_{21}H_{30}O_4$ requires. C, 72 8, H, 8.73, O, 18.64%).

Ozonolysis of nivenolide **1a**. Nivenolide **1a** (450 mg) in MeOH (50 ml) was ozonized at −70° for 25 min. After elimination of the excess of O_3, the MeOH soln was left at room temp. with perhydrol (30%, 5 ml) and aq. KOH (10%, 5 ml) during 6 hr. The reaction mixture was acidified with dil. HCl and extracted with EtOAc The organic soln was washed, dried

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and the solvent removed. The crude crystalline product obtained, was recrystallized from Me_2CO–hexane to give **3** (200 mg), mp 190–196°. The analytical sample showed mp 208–212°, $[\alpha]_D$ +22.43 (2.23, MeOH), ν_{max} 3500, 1710 cm^{-1}, δ. 0.73 (*s*, C-10-Me), 1.17 (*s*, C-4-Me). (Found: C, 64.84; H, 8.16. $C_{16}H_{24}O_5$, requires: C, 64.33; H, 8.15%).

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CASTANOPSIN, A NEW TRITERPENE FROM *CASTANOPSIS INDICA**

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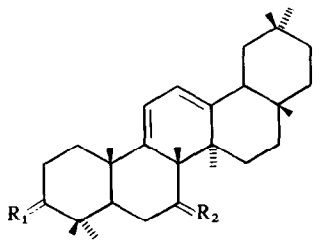
Key Word Index—*Castanopsis indica*; Fagaceae; castanopsin; pentacyclic triterpene; olean-9,12-diene-3β,7α-diol.

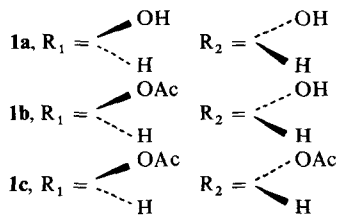
INTRODUCTION

In an earlier communication [1] structure elucidation of two triterpenoids, castanopsone and castanopsol, along with the isolation of castanopsin, another new triterpenoid from *Castanopsis indica* were reported. This paper describes the structure elucidation of castanopsin.

RESULTS

Castanopsin, mp 224–8°, $C_{30}H_{48}O_2$ (M^+ 440), showed the diagnostic colour reactions of unsaturated triterpenoids. The IR spectrum exhibited absorptions for OH (3575, 3290), *gem* dimethyl (1375, 1365) and a conjugated trisubstituted double bond (1630, 835 cm^{-1}). The NMR spectrum showed the signals for eight quaternary methyls in the region δ 0.83–1.2 ppm, two carbinolic protons at 3.78 (*q*) and 4.14 ppm (*t*, $W_{\frac{1}{2}}$ = 7 Hz) respectively and two protons on a *cis*-conjugated double bond as a pair of doublets at 5.53 and 5.71 (J = 6 Hz). The latter was supported by UV absorption (286 nm, log ε 3.9) due to the homoannular diene system in the molecule.





Acetylation of castanopsin at 0° gave a monoacetate (**1b**) mp 184–5°, whose IR spectrum still showed the presence of OH in the molecule. Its NMR spectrum exhibited an acetoxymethyl signal at δ 2.00 ppm and the corresponding geminal proton at 5.02 ppm. When acetylation was carried out at waterbath temperature, castanopsin yielded a diacetate (**1c**) whose IR spectrum was devoid of OH absorption and the NMR spectrum showed two acetoxymethyl signals at δ 2.00 and 2.03 ppm and two carbinolic protons at 4.93 and 5.36 ppm. Thus, the presence of two secondary OH groups in the molecule was confirmed and one of these was slightly hindered in nature.

One OH group (3.78 ppm) was allocated to the C-3 position on biogenetic grounds. The SeO_2 oxidation of castanopsin diacetate yielded a dienedione derivative (**2**) showing UV absorption at 282 nm and IR bands at 1685, 1648 and 1603 cm^{-1}, thus relating castanopsin to a β-amyrin derivative similar to saikogenin B [2]. On the basis of UV absorption and formation of a dienedione the position of the diene chromophore was fixed as $\Delta^{9(11),12(13)}$.

The MS showed a prominent fragment ion at *m/e* 255, derived from rings C, D and E, which eliminated the presence of the hindered hydroxy [3–6] group in rings C, D and E. Thus it could be located at either C-6 or C-7. The $W_{\frac{1}{2}}$ of the geminal proton at δ 4.14 ppm corresponding to the hindered hydroxyl group was of the order of 7 Hz which was compatible with its equatorial configuration and placement at C-7. The relative hindrance to acylation of the axial OH further confirmed this assignment.

The stereochemistry of the OH at C-3 was established from the NMR spectrum. The carbinolic proton at δ 3.78 ppm in castanopsin appeared as a quartet (J_{aa} = 10 Hz, J_{ae} = 6.5 Hz) due to coupling with adjacent methyl-

* CDRI Communication No. 2332.