HETEROCYCLES, Vol. 27, No. 6, 1988

SECONDARY METABOLITES FROM THE ROOTS OF Ligusticum porteri (UMBELLIFERAE). X-RAY STRUCTURE OF Z-6.6',7.3a'-DILIGUSTILIDE [1,2]

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Abstract - Z-Ligustilide (1), riligustilide (2), Z-6.6',7.3a'-diligustilide (7), isovanillin and 4-hydroxy-3-methoxycinnamic acid, were isolated from the roots of Ligusticum porteri C. & R. (family Umbelliferae; common name: chuchupate), which are used in traditional medicine. The chemical characterization of 3,8-dihydro derivative of 7 (9), allowed the structural reassignment of a constituent previously isolated from L. wallichii.

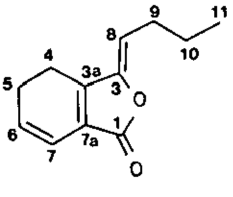
Several plants belonging to the genus Ligusticum (Umbelliferae) are used in traditional medicine.[3,4] In particular, the rootstocks rhizomes of L. wallichii are used in Asia for the preparation of a hot water extract having haemodynamic and analgesic effects. Phthalides have been isolated as the main constituents from this plant.

L. porteri Coulter & Rose (Tarahumara name: wasia; Mexican name: chuchupate; American Indian and Spanish American name: oshá) is well known as traditional medicine in Mexican communities.[5] Among various ethnic groups of Mexico and the adjacent United States, the roots are prepared as an infusion which is drunk to treat gastrointestinal ailments and headaches and as a poultice which is applied to the body to alleviate various pains. Teas and tinctures are consumed as remedies for bronchitis, pneumonia, tuberculosis, colds, coughs and sore throats. As part of our collaborative studies on useful Mexican plants of the Umbelliferae,[6] we have investigated the chemistry of L. porteri, a plant whose natural distribution is limited to the pine-oak forests of the Northern Sierra Madre Occidental, Mexico.

The rootstocks of L. porteri were obtained from Market in Chihuahua City, Chihuahua, Mexico,[7] air dried and exhaustively extracted with n-hexane, chloroform and ethanol. Preliminary comparative chromatographic analysis indicated that the chloroform extract contained the major organic constituents of the plant material, and this residue was chromatographed on an open column of silica gel

(4 x 120 cm) using increasing concentrations of ethyl acetate in n-hexane as solvent system. The residues were handled at low temperatures, protected from light and under Argon atmosphere. The appropriate fractions were further purified by succesive column chromatography and by Prep-TLC. The major metabolite, 1 (isolated in 0.31% yield of the dry weight of the root), was analysed for $C_{12}H_{14}O_2$ by mass spectra and elemental analysis. The compound lacked specific rotation and was recognized to be identical to Z-ligustilide (1) by direct comparison.[8]

Compound 2, (isolated in 0.03% yield of the dry weight of the root), was dimer of 1 as determined by both mass spectrometry and ^{13}C NMR methods, and showed identical spectroscopic properties to those recently reported for riligustilide.[9] The absence of optical activity as well as the centrosymmetric crystal structure found by X-ray analysis[10] gave definitive evidence of the presence of both enantiomers. Catalytic hydrogenation of 2 (H_2, Pd-C 10%, EtOAc, 3 hrs.) afforded a mixture of two compounds which were separated by Prep-TLC (n-hexane:acetone, 7:3, two developments). The more mobile compound is the 6,7,3',8'-tetrahydro derivative 3,[12] since the vinylic signals in the ^1H NMR spectrum were absent and appeared additional signals between δ 2.10-1.30. At δ 4.85 resonates the proton geminal to the lactonic closure (H-3') and the broad doublet (J = 6 Hz) centred at δ 3.33 corresponded to H-7'. The configuration of C-3' of 3 was established as 3'R according to the accesibility for hydrogen from the si face at this centre of the molecule 2. The less mobile product, 4, had the same MW as 2 and showed in the ^1H NMR spectrum complex 4H- signals at δ 7.85-7.35, indicating the presence of aromatic protons. On the other hand, the complex signal centred at δ 4.83 belongs to the proton geminal to the ethereal oxygen of the γ-lactone ring (H-3'). This clearly demonstrates the presence of a 4,5-dehydro-3',8'-dihydro derivative of 2.[13] The R configuration at C-3' of 4 was established for the same reason described above for the stereochemistry of 3. These results were analogous to those obtained for the reduction of angeolide (5),[14] and were attributed to the high degree of steric shielding of the spiro lactone system present in both compounds 2 and 5.



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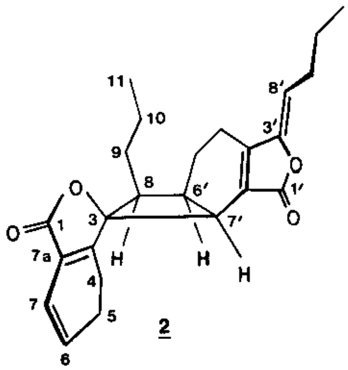

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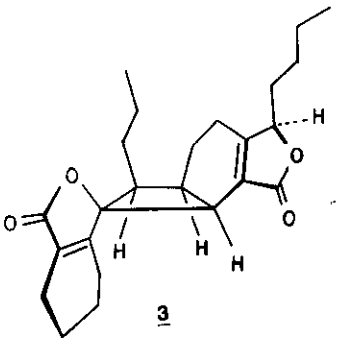

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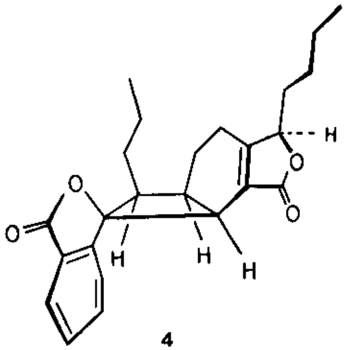

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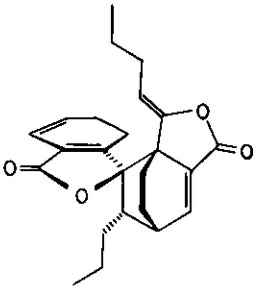

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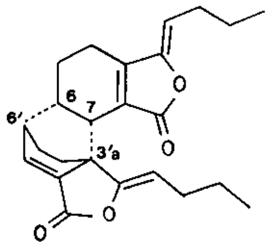
The natural product 7 (0.03% yield), mp 123°C, was also a dimeric phthalic lactone, as demonstrated by mass spectrometry and ^{13}C NMR. The spectroscopic data are consistent with those published for Z,Z'-6.6',7.3a'-diligustilide 6, a Diels Alder adduct of Z-ligustilide (1) isolated previously.[9] Direct comparison of the substances from *L. wallichii* and *L. porteri* kindly provided by Professor Kaouadji, confirmed their identity of both. The already published formula 6,[9,15] does not clearly show the stereochemistry at C6' and C3a'. However, the identity of 7 with levistolide A isolated from *Levisticum officinale* (levistolide A)[16] suggests the *endo* (rather than *exo*) orientation of the Diels-Alder adduct, as depicted in formula 7.



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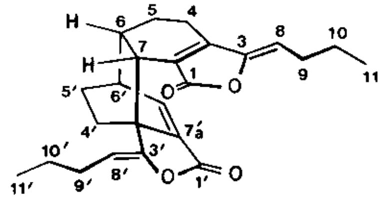

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Final proof for the structure and stereochemistry of Z,Z'-6.6',7.3a'-diligustilide 7, was obtained by single X-ray diffraction. The absence of optical activity indicated the presence of racemic compound. Figure 1 is a computer perspective drawing of an enantiomer of 7.[17]

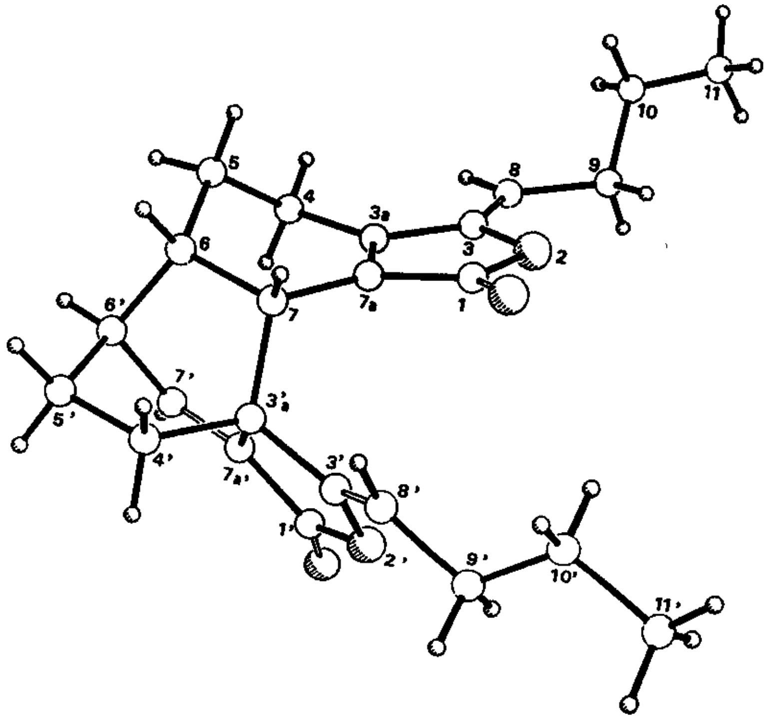
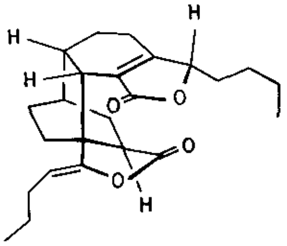


Figure 1.
Computer - generated
pespective drawing of 7

Hydrogenation of a solution of 7 in ethyl acetate over 10% Pd-C for 24 h, gave a mixture of two products which were separated three times by Prep-TLC, eluting with n-hexane-acetone, 7:3, 7:3 and 4:1, respectively. The minor (10% yield) and more polar compound is the 3,8,7',7a'-tetrahydro derivative 8,[20] since the signals of the vinylic protons H-7 and H-8 disappeared and the proton geminal to the γ-lactone resonates at δ 4.52 (H-3, $w_{1/2}$ = 12 Hz). The IR absorptions at 1792 and 1753 cm^{-1} gave evidence for an enolic γ-lactone and an α,β-unsaturated γ-lactone, respectively, in agreement with formula 8. The S configuration at C-3 and R at C-7a' were established according to the less hindred approach of hydrogen to the C-3 re and C-7a' si faces of the depicted enantiomer of 7. The major (70% yield) and less polar compound, 9,[21] showed the signals for H-7' at δ 7.29 and H-8' at δ 4.54. The saturation of the C3-C8 double bond (instead of C3'-C8') was confirmed by the disappearance of the UV absorption at 273 nm, due to the $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl chromophore. The high steric shielding around the C3'-C8' double bond does not allow its saturation under these reaction conditions.

The structure 9 (without the stereochemistry at C-3) was recently assigned as Z'-3,8-dihydro-6.6', 7.3a'-diligustilide, an oily dimeric phthalide derivative isolated from *L. wallichii*.[9] However, to our surprise, in addition to the differences in physical states, comparison of spectroscopic data showed quite different values and clearly indicate that our dihydro derivative 9 and the natural product of the French workers are different compounds. In particular, the proposed assignments for

H-3 (δ 5.11) and H-8' (δ 5.18)[9] were at low field with respect ours (H-3: δ 4.54; H-8': δ 4.96).[21] Furthermore, the reported λ_{max} at 273 nm (due to a conjugated dienone chromophore) is not consistent with formula 9, and therefore the proposed structure for the natural product is in error. Analysis of the published data[9] led to the deduction that the constituent from *L. wallichii* is *rel* (3'*R*)-*Z*-3',8'-dihydro-6.6',7.3a'-diligustilide 10, since this structure is in accord with (a) λ_{max} at 273 nm, (b) the signal at δ 5.18 corresponds to H-8, consistent with a δ proton of a conjugated dienone, (c) the signal at δ 5.11 is assigned to H-3' and the configuration at C-3 in the depicted enantiomer is *R*, since H-3 is deshielded by the carbonyl group at C-1, and (d) the diamagnetic shift of H-7 of 9 (δ 3.02) with respect to that of 7 (δ 3.27) is also in agreement with the saturation of the C3'-C8' double bond. Therefore, structure 10 is the natural product from *L. wallichii*.[22]



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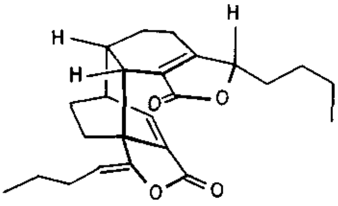

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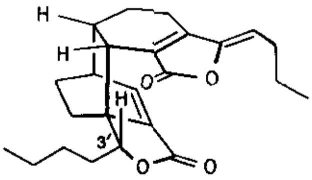

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Finally, from the more polar eluates of the main column chromatography traces of isovanillin and 4-hydroxy-3-methoxycinnamic acid were isolated and identified by direct comparison with authentic samples.

An extremely interesting feature of the dimeric phthalides isolated from this group of plants is that they are found as racemic mixtures.[23]

The pharmacological properties of the compounds isolated from *L. porteri* are being studied under the auspices of the International Organization of Chemistry for Development (IOCD). In order to meet the increasing demand for this medicinal herb, a biological study funded by the Agency for International Development (AID) of the United States has begun cultivating this plant which is threatened with local extinction due to overcollecting for commercial medicine purposes.

ACKNOWLEDGEMENTS

We thank Dra. Rachel Mata, Facultad de Química de la UNAM, for encouragement in the course of this research, and Messrs. Jorge Cárdenas, Rubén Gaviño, Luis Velasco, Misael Torres and René Villena of the Instituto de Quimica de la UNAM, for the measurements of the spectra. We also wish to express our thanks to Prof. Mourad Kaouadji for direct comparison of 7, and to Prof. Yoshiyasu Fukuyama and Prof. Leonard F. Bjeldanes for spectroscopic data of some phthalides. Special acknowledgement is due to IOCD for partial support.

REFERENCES AND NOTES

1. This paper is dedicated to Prof. Pierre Crabbé, deceased on August 7, 1987.
2. Contribution No. 893 from the Instituto de Quimica de la UNAM.
3. M. J. M. Gijbels, J. J. C. Scheffer and A. B. Suendin, *Rev. Ital. EPPOS*, 1979, 51, 335.
4. *The Chinese Pharmacopeia*, 1963, 30.
5. R. A. Bye Jr., *Economic Bot.*, 1986, 40, 103; R. A. Bye Jr., in *Two Mummies from Chihuahua, Mexico*, (R. A. Tyson and D. V. Elerick, Eds.) *San Diego Museum Papers*, 1985, No. 19, pp. 77; R. A. Bye Jr. and E. Linares, *J. Ethnobiology*, 1986, 6, 289.
6. G. Delgado and J. Garduño, *Phytochemistry*, 1987, 26, 1139; E. Linares and R. A. Bye Jr., *J. Ethnopharmacology*, 1987, 19, 153.
7. Roots collected after the growing season in the Sierra Tarahumara, Chihuahua, Mexico, were obtained in the La Reforma market of Chihuahua City (4 Oct. 1985). The voucher specimen (R. Bye and E. Linares 14148) is deposited in the ethnobotanical collection, a satellite of the Herbario Nacional (MEXU), of the Instituto de Biología de la Universidad Nacional Autónoma de México. The identity was confirmed by morphological comparison with flowering and fruiting specimens collected in the same area by R. B. and E. L.
8. M. J. M. Gijbels, J. J. C. Scheffer and A. Baerheim Svendsen, *Planta Medica*, 1982, 44, 207.
9. M. Kaouadji, F. De Pachtere, C. Pouget, A. J. Chulia and S. Lavaitte, *J. Nat. Prod.*, 1986, 49, 872.
10. An independent[11] X-ray analysis was performed on 2. The crystal of 2, 0.28 x 0.22 x 0.44 mm, monoclinic $P2_1/c$, a=6.097 (3), b=12.753 (7), c=27.285 (12), β=92.73 (4)°, Dc = 1.19 g cm^{-3}, μ (MoKα) = 0.75 cm^{-1}, Z = 4, converged to R = 0.0882 (R_ω = 0.099) for 2001 unique observed reflections of 3725 reflections collected. Data structure, structure solution and refinement are the same as in reference 17. Figure 2 is a computer perspective drawing of an enantiomer of 2.
11. Previous crystallographic characterization of 2 was published: Y. Meng, O. Wang, H. Zhang, Y. Chen, Y. Fan and F. Wang, *Lanzhou Daxue Xuebao, Ziran Kuxueban*, 19, (Huaxue Jikan) 76 (1983). (*Chem. Abst.*, 1984, 100, 135781j).
12. (*Z*)-*rel*-(8*S*, 6'*R*, 7'*S*, 3'*R*)-6,7,3'-8'-Tetrahydro-8.6',3.7'-Diligustilide 3. Colorless oil; λ_{max} (MeOH)=235 nm (ϵ 15972); IR ($CHCl_3$) cm^{-1}: 3028, 3014, 2959, 2935, 2868, 1746, 1673, 1072, 1039, 940. ^1H NMR (80 MHz, $CDCl_3$): δ 4.85 (1H, *m*, $W_{1/2}$ = 13 Hz, H-3'), 3.33 (1H, br *d*, $J_{6'-7'}$ =

6 Hz, H-7'), 2.84 (1H, *t*, $J_{6'-8}$ = 7 Hz, H-6'); EIMS (70 eV, rel. intensity): 384 (M^+, 0.6), 193 (79), 192 (100), 163 (18), 150 (15).

13. (*Z*)-*rel*-(8*S*, 6'*R*, 7'*S*, 3'*R*)-4,5-dehydro-3',8'-dihydro-8.6',3.7'-Diligustilide 4: Colorless oil; λ_{max} (MeOH: 223 nm, ε 14822; 276 nm, ε 2107); IR ($CHCl_3$) cm^{-1}: 3031, 3016, 2960, 2933, 2873, 1757, 1085, 953; ^{1}H NMR (80 MHz, $CDCl_3$): δ 7.85-7.35 (4H, complex signal due to benzenoid protons), 4.83 (1H, *m*, $W_{1/2}$ = 13 Hz, H-3'), 3.56 (1H, br *d*, $J_{6'-7'}$ = 9 Hz, H-7'); EIMS (70 eV, rel. intensity): 380 (M^+, 17), 193 (100), 188 (35), 159 (42), 146 (24).

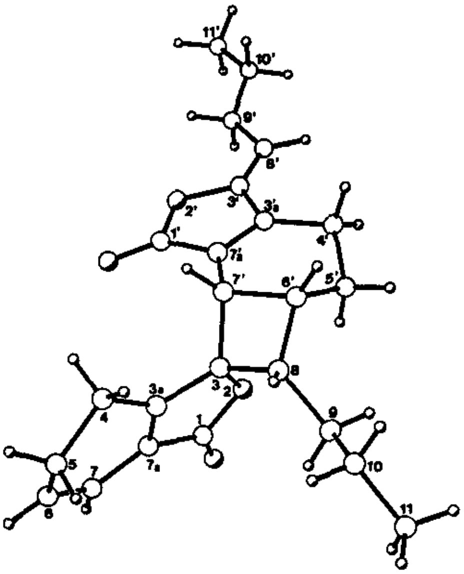


Figure 2.
Computer - generated
perspective drawing of 2

14. S. K. Banerjee, B. D. Gupta, W. S. Sheldrick and G. Höfle, *Liebigs Ann. Chem.*, 1982, 699.

15. M. Kaouadji, H. Reutenauer, A. J. Chulia and A. Marsura, *Tetrahedron Lett.* 1983, *24*, 4677; M. Kaouadji, A. M. Mariotte and H. Reutenauer, *Z. Naturforsch.* 1984, *39c*, 872.

16. M. Cichy, V. Wray and G. Höfle, *Liebigs Ann. Chem.*, 1984, 397.

17. Crystals of Z-6.6',7.3a'-diligustilide 7 were obtained by slow crystallization from ethyl acetate. The crystal was monoclinic, space group $P2_1/n$ with a=9.308 (4), b=8.356 (2), c=26.378 (13) Å, β=93.67 (4)°, and d_{calc} = 1.23 g cm^{-3}. The data were measured on a Nicolet R3m diffractometer (graphite monochromated MoKα radiation). The size of the crystal used was approximately 0.4 x 0.4 x 0.4 mm, μ=0.77 cm^{-1}. A total of 3592 accesible reflections were measured on ω scan mode for 2θ≤50° of which 2257 were considered to be observed |Fo|≤3 (Fo). The structure was solved by direct methods and refined by block diagonal least-squares with anisotropic temperature factor for non-hydrogen atoms and fixed isotropic temperature factor U=0.06Å for H-atoms. The final R value was 0.089 (R_ω = 0.099). Atomic scattering factors from Inter-

national Tables for X-ray Crystallography. All calculations were performed on a NOVA 45 computer using SHELXTL package program.[18,19]

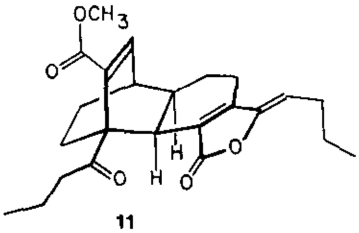
18. G. M. Sheldrick, SHELXTL Revision 3. An integrated system for solving, refining and displaying structures from diffraction data. University of Göttingen, Federal Republic of Germany, 1981; International Tables for X-Ray Crystallography. Vol. IV, 1974. Kynoch Press, Birmingham, p. 99-101.

19. Listing of pertinent crystallographic data are available on request to the senior author.

20. (Z')-rel-(3S, 6S, 7S, 7a'R)-3,8,7',7a'-Tetrahydro-6.6',7.3a'-Diligustilide 8: Mp 110-113°C (from EtOAc); λ_{max} (MeOH: 211 nm (ε 11327); IR ($CHCl_3$) cm^{-1}: 2957, 2932, 2872, 1792, 1753, 1666, 1463, 1174, 1061, 1022, 1000, 980; ^1H NMR (80 MHz, $CDCl_3$): δ 4.68 (1H, t, J = 8 Hz, H-8'), 4.52 (1H, m, $W_{1/2}$ = 12 Hz, H-3), 2.86 (1H, br d, $J_{6,7}$ = 10 Hz, H-7); EIMS (70 eV, rel. intensity); 384 (M^+, 100), 356 (37), 327 (38), 189 (32), 187 (31), 163 (81), 107 (47), 105 (32), 91 (61), 83 (42), 79 (66), 77 (46), 55 (79), 41 (42).

21. (Z,Z')-rel-(3S, 6S, 7S)-3,8-Dihydro-6.6',7.3a'-Diligustilide 9: Mp. 123°C (from EtOAc); λ_{max} (MeOH) = 202 nm (ε 11479); IR ($CHCl_3$) cm^{-1}: 2959, 2933, 2872, 1754, 1710, 1666; ^1H NMR (80 MHz, $CDCl_3$): δ 7.29 (1H, br d, $J_{7'-6'}$ = 7 Hz, H-7'), 4.96 (1H, t, J = 8 Hz, H-8'), 4.54 (1H, m, $W_{1/2}$ = 12 Hz, H-3), 3.18 (1H, br d, J = 9 Hz, H-7), 2.96 (1H, ddd, J = 6.5, 2.5, 2.5 Hz, H-6'); EIMS (70 eV, rel. intensity): 382 (M^+, 2.9), 193 (51), 190 (100), 161 (45), 148 (54), 107 (25), 105 (28), 91 (25), 79 (24), 77 (20), 55 (51), 41 (28).

22. Wallichilide (11), a product that may be derived by methanolisis of 7, has been reported as a natural product from a Japanese collection of L. wallichii: W. Pushan, G. Xuanling, W. Yixiong, Y. Fukuyama, I. Miura and M. Sugawara, Phytochemistry, 1984, 23, 2033.



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23. Diterpenoid compounds from both antipodal stereochemical series have been detected within a single species, e.g.: D. E. U. Ekong and J. I. Okugun, Chem. Commun., 1967, 72.

Received, 25th December, 1987