*Journal of Natural Products*
*Vol. 46, No. 5, pp. 675-680, Sep-Oct 1983*



# DAPHNANE DITERPENES FROM *WIKSTROEMIA MONTICOLA*: WIKSTROTOXINS A-D, HURATOXIN, AND EXCOECARIATOXIN

SHIVANAND D. JOLAD, JOSEPH J. HOFFMANN, BARBARA N. TIMMERMANN, KARL H. SCHRAM, JACK R. COLE,*

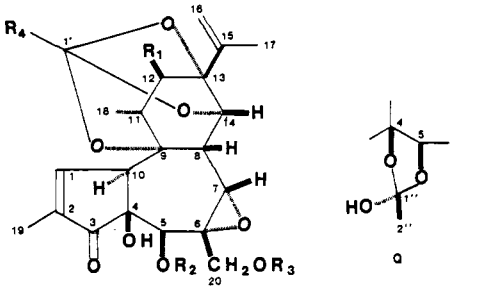
*College of Pharmacy*

ROBERT B. BATES, ROBERT E. KLENCK, and MICHAEL S. TEMPESTA

*Department of Chemistry, University of Arizona, Tucson, AZ 85721*

ABSTRACT.—An ether extract of *Wikstroemia monticola* (Thymelaeaceae) yielded, upon further fractionation, a tlc-homogeneous mixture. The resolution of this mixture by reversed phase hplc gave six daphnane diterpenes: wikstrotoxins A (**1**), B (**3**), C (**5**), and D (**6**), huratoxin (**2**), and excoecariatoxin (**4**). The identities of wikstrotoxins A-D, which are new, were established by spectral properties. For the first time, $^{13}$C-nmr data are given for **2**.

Daphnane diterpenes occur in the families Euphorbiaceae and Thymelaeaceae (1). During the course of our continuing search for plants having tumor-inhibitory constituents, an ether extract of *Wikstroemia monticola* Skotsberg (Thymelaeaceae) gave, upon further fractionation, a tlc single-spot fraction, which, when subjected to reversed phase hplc, yielded six daphnane diterpenes: wikstrotoxins A (**1**), B (**3**), C (**5**), and D (**6**), huratoxin (**2**), and excoecariatoxin (**4**). Huratoxin (**2**) from *Hura crepitans* (2-4) and excoecariatoxin (**4**) from *Excoecaria agallocha* (5), both from the family Euphorbiaceae, had been previously characterized. As $^{13}$C-nmr spectral data on **2** have not been reported, they are included herein (table 1).



Q

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | n |
|---|---|---|---|---|---|
| **1** | H | H | H | $-(CH_2)_nMe$ | 9 |
| **1a** | H | Ac | Ac | | 9 |
| **2** | H | H | H | | 8 |
| **2a** | H | Ac | Ac | | 8 |
| **3** | H | H | H | | 6 |
| **4** | H | H | H | | 4 |
| **5** | OAc | Q | H | | 8 |
| **6** | H | H | H | $-(CH_2)_8Me$ | |

## DISCUSSION

The structures of wikstrotoxins A-D (**1, 3, 5,** and **6**) were deduced from spectral evidence by comparing one with another and with huratoxin (**2**) and excoecariatoxin (**4**). Wikstrotoxin B (**3**) was not obtained in pure form, but because its identification in the presence of another component posed no serious problem, no further attempts were made to purify it. All of these compounds, except **5**, have essentially the same $^{1}$H-nmr

TABLE 1. $^{13}$C-nmr chemical shifts (δ) for Huratoxin (**2**) and Wikstrotoxin D (**5**) in $CDCl_3$

| C-atom | Compound **2** | Compound **5** |
|---|---|---|
| 1 | 161.2 d | 160.4 |
| 2 | 136.7 s | 136.9 |
| 3 | 209.8 s | 209.4 |
| 4 | 84.4 s | 83.7 |
| 5 | 82.0 d | 80.4 |
| 6 | 60.5 s | 60.4 |
| 7 | 48.2 d | 47.5 |
| 8 | 36.7 d | 36.7 |
| 9 | 72.3 s | 72.2 |
| 10 | 64.2 d | 67.3 |
| 11 | 34.9 d | 35.4 |
| 12 | 36.5 t | 44.0 d |
| 13 | 79.6 s | 78.2 |
| 14 | 71.9 d | 72.1 |
| 15 | 146.2 s | 143.1 |
| 16 | 111.2 t | 113.3 |
| 17 | 20.4 q | 18.7 |
| 18 | 9.9 q | 9.9 |
| 19 | 18.9 q | 18.2 |
| 20 | 65.0 t | 65.1 |
| 1′ | 116.5 s | 117.0 |
| 2′ | 138.8 d | 139.3 |
| 3′-5′ | 122.9, 128.8, 134.7 d | 122.3, 128.6, 135.1 |
| 6′ | 32.7 t | 32.7 |
| 7′-11′ | 29.1, 29.1, 29.3, 29.5, 29.6 t | 29.1, 29.1, 29.3, 29.5, 29.7, |
| 12′ | 31.9 t | 31.9 |
| 13′ | 22.7 t | 22.7 |
| 14′ | 14.1 q | 14.1 |
| Me*C*=0 | — | 169.6 s |
| *Me*C=0 | — | 21.1 q |
| 1″ | — | 111.4 s |
| 2″ | — | 29.5 q |

parameters (table 2) for all groupings except for those in the side chain at C-1′, and thus, they differ only in this side chain. Compounds **1-4** show the *E,E*-2′,4′-diene pattern characteristic of huratoxin (**2**), and differ only in the number of methylenes further out in the chain. The finding of chains with four, six, and eight methylenes (in **4, 3,** and **2,** respectively) is not surprising, but the presence of nine methylenes (in **1**) is biogenetically unusual. Compound **6** has a saturated 10-carbon chain not found in an orthoester of this type but observed in 12-ester and 12,20-diester daphnane diterpenes.[1]

Wikstrotoxin C (**5**) shows significant differences from the other compounds in its $^1$H-nmr shifts for protons attached to carbons 7, 8, 11, 14, 18, and especially 12 (which moves downfield 3 ppm and becomes a singlet), and shows singlets for two additional methyl groups at δ 1.83 and 1.99. It is clearly a 12β acetate, like many phorbol esters (1), with the δ 1.99 peak being due to the acetate methyl group, and an essentially zero coupling constant between the protons on carbons 11 and 12 due to the approximately 90° dihedral angle between them. The other methyl group (δ 1.83) absorbs rather far upfield for an acetate grouping, and we think it to be in a hemiorthoester grouping, which can only involve the C-4 and C-5 hydroxyl groupings, as the C-20 absorptions are unchanged. The new chiral center that results probably has the configura-

[1]See reference (1), Notes 37, 90, and 91.

TABLE 2. $^1$H-nmr chemical shifts (δ) and coupling constants (Hz, in parentheses) for **1-6** and **2a** in $CDCl_3$

| Proton | 1 | 2 | 2a | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 1 | 7.63 bs | 7.61 | 7.54 | 7.64 | 7.64≈s | 7.57 | 7.60 dq (2.4, 1.3) |
| 5 | 4.25 s | 4.26 | 5.55 | 4.25 | 4.26 | 4.25 | 4.25 |
| 7 | 3.45 s | 3.44 | 3.36 | 3.45 | 3.45 | 3.55 | 3.43 |
| 8 | 2.94 d (2.3) | 2.93 (2.4) | 3.00 (2.6) | 2.94 (2.5) | 2.95 (2.0) | 3.51 (2.4) | 2.90 (2.4) |
| 10 | 3.82 d (2.8) | 3.82~s | 3.95~p (2.5) | 3.81 (2.8) | 3.81 d (2.9) | 3.83~s | 3.74 d (2.4) |
| 11 | 2.49 p (7.0) | 2.48~p (7.0) | 2.40 (7.3) | 2.40 (7.2) | 2.49 (7.3) | 2.37 q (7.3) | 2.45~p (7.7) |
| 12α | 1.68 d (14.2) | 1.65 (14.0) | 1.68 (14.3) | 1.68 (14.1) | 1.68 (14.0) | 4.97 s | 1.64 d (14.3) |
| 12β | 2.23 dd (14.2, 8.6) | 2.33 (14.3, 8.6) | 2.23 (14.3, 8.6) | 2.23 (14.1, 8.6) | 2.24 (14.0, 8.6) | — | 2.21 (14.3, 8.7) |
| 14 | 4.43 d (2.3) | 4.44 (2.4) | 4.43 (2.6) | 4.43 (2.5) | 4.43 (2.0) | 4.76 (2.4) | 4.37 (2.4) |
| 16 | 4.90 s, 5.02 s | 4.90, 5.02 | 4.92 t (1.4), 5.03 | 4.91, 5.02 | 4.91, 5.03 | 4.95, 5.01 | 4.89, 5.02 |
| 17 | 1.79 s | 1.79~s | 1.80 | 1.79 | 1.80 | 1.79 | 1.78 |
| 18 | 1.18 d (7.0) | 1.17 (7.0) | 1.13 (7.0) | 1.18 (7.1) | 1.18 (7.3) | 1.29 (7.3) | 1.16 (7.1) |
| 19 | 1.79 s | 1.79~s | 1.77 dd (2.5, 1.3) | 1.79 | 1.80 | 1.79 | 1.80 d (1.3) |
| 20 | 3.79 d (12.5), 3.86 d (12.5) | 3.79 (12.3), 3.84 (12.3) | 3.60 (11.9), 4.69 (11.9) | 3.78 (12.6), 3.86 (12.7) | 3.79 (12.7), 3.87 (12.7) | 3.79 (12.5), 3.92 (12.5) | 3.78 (12.2), 3.86 (12.2) |
| 2′ | 5.70 d (15.4) | 5.70 (15.4) | 5.71 (15.4) | 5.70 (15.5) | 5.70 (15.4) | 5.64 (15.4) | 1.95 m |
| 3′ | 6.70 dd (15.4, 10.5) | 6.70 (15.4, 10.3) | 6.71 (15.4, 10.5) | 6.70 (15.4, 10.5) | 6.70 (15.4, 10.5) | 6.66 (15.4, 10.3) | 1.60~p |
| 4′ | 6.05 dd (15.1, 10.5) | 6.05 (15.6, 10.3) | 6.06 (15.6, 10.5) | 6.05 (15.0, 10.5) | 6.05 (15.0, 10.5) | 6.04 (15.0, 10.3) | |
| 5′ | 5.84 dt (15.1, 6.9) | 5.85 (15.6, 6.8) | 5.85 (15.6, 7.1) | 5.85 (15.0, 6.9) | 5.85 (15.0, 7.0) | 5.85 (15.0, 7.0) | |
| 6′ | 2.09 q (6.9) | 2.09 (6.8) | 2.10 (7.2) | 2.09 (7.0) | 2.10 (7.0) | 2.09 (7.0) | ~1.26 |
| 7′ | 1.37~p (7.0) | 1.37 m | 1.37 | 1.38~p (~7.0) | 1.39 (~7.0) | 1.37 m | |
| 8′-(n+5)′ | 1.26~s | 1.26 | 1.27 | 1.26 | 1.29 m | 1.26~s | |
| (n+6)′ | 0.88 t (6.6) | 0.88 (6.4) | 0.88 (6.6) | 0.88 (6.6) | 0.88 (7.0) | 0.88 (6.6) | 0.88 (6.6) |
| MeC=O | | | 2.02, 2.19 s | | | 1.99 s | — |
| 2″ | — | — | — | — | — | 1.83 s | — |

tion shown in Q to permit hydrogen bonding to the C-3 carbonyl oxygen. Hemiorthoesters have been found previously in terpene derivatives (6), and there is supporting evidence from the $^{13}$C-nmr spectrum (table 1) showing a peak for the quaternary carbon in the hemiorthoester grouping at δ 111.4. This is the first daphnane diterpene with a hemiorthoester grouping.

The mass spectra of **1, 3,** and **4** were very similar to one another and to that of huratoxin (**2**). They all displayed discernible molecular ion peaks at *m/z* 598 (**1**), 584 (**2**), 556 (**3**), and 528 (**4**), followed by peaks corresponding to the loss of $H_2O$, $CH_2OH$, and ($H_2O$+$CH_2OH$). These peaks were seen clearly in the mass spectrum of the tlc single-spot mixture and after acetylation with appropriate peak shifts. The numbers of methylene groups in the side chains were observed clearly from the prominent peaks at *m/z* 221 (**1**), 207 (**2**), 179 (**3**), and 151 (**4**), corresponding to $R_4CO^+$, not shifted in the mixture of acetates. The diterpene moiety after the loss of $R_4COOH$ was seen more directly as a peak at *m/z* 360 and its fragmentation peaks with appropriate peak shifts in the acetate mixture. The elemental composition of all peaks corresponding to the diacetates **1a** and **2a** shown in figure 1 were verified by high-resolution exact-mass measurements.

The molecular ion peaks of **5** (*m/z* 684) and **6** (*m/z* 532) were not observed in the mass spectrum of the tlc single-spot mixture nor in its acetylation products, but were clearly seen in their mass spectra after purification. Their spectra were similar to those of **1-4,** with peaks at *m/z* 207 (**5**) and 155 (**6**) corresponding to $R_4CO^+$. Additional peaks at *m/z* 642 (M-$COCH_2$), 624 (M-AcOH), 612 [M-($COCH_2$+$CH_2O$)], 607 [M-(AcOH+OH·)], 593 [M-(AcOH+$COCH_2$)], 582 [M-(AcOH+$COCH_2$)], 570 [M-(2 x $COCH_2$+$CH_2O$)], 564 [M-(2 x AcOH)], 460 [M-$C_{14}H_{24}O_2$ (side chain at C-1′)], 418 [M-($C_{14}H_{24}O_2$+$COCH_2$)], 400 [M-($C_{14}H_{24}O_2$+AcOH)], 376 [M-($C_{14}H_{24}O_2$+2 x $COCH_2$)], 375 [M-($C_{14}H_{24}O_2$+$COCH_2$+Ac·)], 359 [M-($C_{14}H_{24}O_2$+$COCH_2$+OAc·)], 358 [M-($C_{14}H_{24}O_2$+$COCH_2$+AcOH)], 341 [M-($C_{14}H_{24}O_2$+AcOH+OAc·)], and 340 [M-($C_{14}H_{24}O_2$+2 x AcOH)] were observed in **5**.

## EXPERIMENTAL[2]

Dried, woody stems and bark of *W. monticola*, collected in Maui, Hawaii, in November 1980, were chopped and then ground to 3-mm particle size and stored at −10° prior to extraction. In a typical run, the ground material (7 kg) was suspended in ether (~35 liters) with occasional stirring for 96 h and filtered, then the filtrate was dried under vacuum. The dried residue (84 g) was taken up in fresh ether (500 ml), and the resulting semi-solid residue that separated was removed by decantation. This semi-solid residue was washed with ether (2 x 100 ml), and the combined ether-soluble fraction was left in the freezer overnight, then filtered, and the filtrate was evaporated to dryness under vacuum. The resulting ether-soluble residue (34 g) was taken up in isopropyl ether (300 ml), stirred for 3 h and filtered, and the filtrate was freed from solvent under vacuum. The isopropyl ether-soluble residue (25 g) was distributed between petroleum ether (500 ml) and 20% aqueous MeOH (500 ml). After extracting the petroleum ether layer with more aqueous MeOH (2 x 100 ml), the combined aqueous MeOH layers were evaporated to dryness under vacuum, and the resulting residue (6.5 g) was subjected to two-funnel partitioning between benzene-MeOH-$H_2O$ (8:5:1, 200 ml each phase). The combined lower phases (3.6 g), after being freed from solvent under vacuum, were subjected to EM $SiO_2$-60 (100 g) column chromatography, eluting with $CH_2Cl_2$ (100%) and then $CH_2Cl_2$ with gradually increasing concentrations of EtOAc. Fractions showing the presence of daphnane diterpenes on tlc were combined and dried under vacuum, and the resulting residue (320 mg) was subjected to preparative tlc (EM $SiO_2$-60 PF-254). Single development with $CH_2Cl_2$-EtOAc (70:30) and work-up followed by decolorization and evaporation of the solvent under vacuum gave a nearly colorless foam (120 mg), homogeneous by tlc with several solvent systems.

Reverse phase tlc of the above material as well as its acetate (prepared by treatment with $Ac_2O$-pyridine at 25°C overnight) showed that it was a mixture of several components, huratoxin (**2**) being the major one. This mixture was resolved by preparative hplc, first on a Waters' PrepLC/System 500A

[2]For general procedures used, see: S.D. Jolad, J.J. Hoffmann, K.H. Schram, J.R. Cole, M.S. Tempesta, and R.B. Bates, *J. Org. Chem.*, **46**, 4267 (1981).

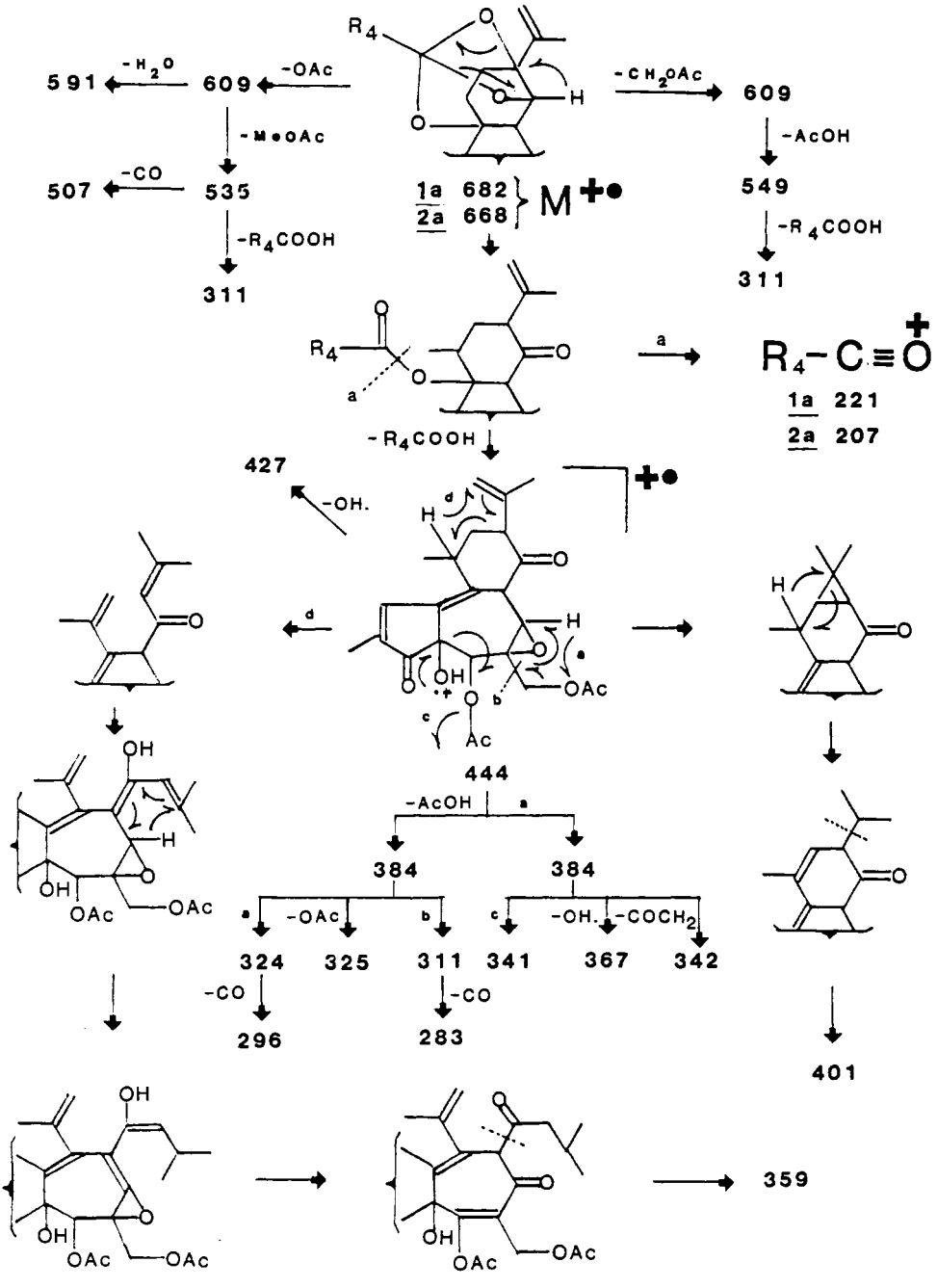



FIGURE 1. Major significant ions (*m/z* ratios) in the mass spectra of **1a** and **2a**.

equipped with a PrepPAK/500/$C_{18}$ column using MeOH-$H_2O$ (85:15) which gave **4**, followed by a mixture of **3** and **6**, then a mixture of **2, 5,** and **6**, then **2,** and finally **1**. Compounds **1, 2, 4,** and the mixture of **2, 5,** and **6** were further chromatographed on a Spectra Physics 3500 fitted with a Whatman's M9, 10/50 ODS-2 $C_{18}$ column using MeOH-$H_2O$ (88:12) to give **1, 2, 4, 5,** and **6** in >95% purity, as judged by analytical reversed phase hplc and $^1$H-nmr.

The tlc single spot mixture demonstrated an activity of 158% (T/C) at 75 μg/kg in the *in vivo* 3PS tumor system. This mixture and purified huratoxin (**2**) are undergoing additional testing for antitumor properties by the National Cancer Institute.

ACKNOWLEDGMENTS

We express our appreciation to Peter Baker for mass spectral data and Pramuk Shivanonda for technical assistance. This investigation was supported by Grants No. 5-R01-CA29626 and No. 2-R01-CA22336-04, awarded by the National Cancer Institute, Department of Health, Education, and Welfare, Bethesda, MD 20014, whom we gratefully acknowledge.

LITERATURE CITED

1. F.J. Evans and C.J. Soper, *J. Nat. Prod.*, **41**, 193 (1978) and references cited therein.
2. K. Sakata, K. Kawazu, and T. Mitsui, *Agr. Biol. Chem.*, **35**, 1093 (1971).
3. K. Sakata, K. Kawazu, T. Mitsui and N. Masaki, *Agr. Biol. Chem.*, **35**, 2113 (1971).
4. K. Sakata, K. Kawazu, T. Mitsui and N. Masaki, *Tetrahedron Lett.*, 1141 (1971).
5. H. Ohigashi, H. Katsumata, K. Kawazu, K. Kashimizu and T. Mitsui, *Agr. Biol. Chem.*, **38**, 1093 (1974).
6. S.D. Jolad, J.J. Hoffmann, K.H. Schram, J.R. Cole, M.S. Tempesta and R.B. Bates, *J. Org. Chem.*, **46**, 641 (1981).

*Received 1 November 1982*