Reaction of 2'-Hydroxychalcone with *N*-Bromosuccinimide. Formation of 3',5'-Dibromo-2'-hydroxychalcone (6a). Method A. To the chalcone **5a** (0.56 g) in dry benzene (20 mL) was added NBS (0.45 g) in an equimolar ratio. The contents were refluxed for 1 h on a water bath and allowed to cool to room temperature. The residue, after evaporation of the benzene, was washed with a little hot water to remove the succinimide. It afforded orange yellow needles of 3',5'-dibromo-2'-hydroxychalcone (**6a**) from ethanol (0.25 g, 26%), mp 143–144 °C (lit.[9,10] mp 143–144 and 145 °C). TLC showed a single spot with a benzene–petroleum ether (30:70) mixture. Anal. Calcd for $C_{15}H_{10}Br_2O_2$: C, 47.2; H, 2.6. Found: C, 46.8; H, 2.4.

The reactions of 2'-hydroxy-4-methoxychalcone (**5b**), 4-chloro-2'-hydroxychalcone (**5c**), and 2'-hydroxy-4-methoxy-5'-methylchalcone (**5d**) with NBS under the same conditions afforded **6b**, **6c**, and **6d**, respectively.

Reaction of 2'-Hydroxychalcone with Pyridine Perbromide. Formation of 3',5'-Dibromo-2'-hydroxychalcone (6a). Method B. The chalcone **3a** (0.6 g) was dissolved in acetic acid (15 mL); pyridine·perbromide (0.6 g) was added in small amounts, keeping the solution at 40–60 °C. The reaction mixture was kept at the same temperature for an additional 30 min and then allowed to stand at room temperature. After 5 days, it was diluted with water; the solid was filtered off and washed with water. Crystallization from ethanol gave orange yellow needles (0.36 g, 36%) of **6a**, mp 143–144 °C, identical in all respects with authentic material.[9,10]

Under similar reaction conditions **5b** (0.6 g), **5c** (0.8 g), and **5d** (0.7 g) reacted with pyridine perbromide to give **6b** (0.4 g, 41%), **6c** (0.46 g, 36%), and **6d** (0.47 g, 52%), respectively (see Table I).

Reaction of 2'-Hydroxychalcone with Pyridine–Bromine Complex. Bromine (0.5 mL) was added to pyridine (15 mL). To this solution was added the chalcone **3a** (2.24 g) in small quantities with shaking at room temperature. After 5 min the separated hydrobromide salt (0.36 g) was filtered off, washed with ether, and dried, mp 213–214 °C (lit.[11] mp 200 °C). The mother liquor was diluted with water and then acidified with dilute HCl. An oily compound that separated gave orange yellow needles (2.4 g, 63%) of **6a**, mp 143–144 °C from ethanol. No depression was observed in mixture melting point with the product obtained from the previous reaction.

By the same procedure **6b** (2.3 g, 57%), **6c** (2.35 g, 58%), and **6d** (1.5 g, 83%) were obtained from **5b** (2.5 g), **5c** (2.5 g), and **5d** (1.4 g), respectively.

Synthesis of 3',5'-Dibromo-2'-hydroxy-4-methoxychalcone Dibromide (3g). 3',5'-Dibromo-2'-hydroxy-4-methoxychalcone (**6b**, 1 g) was dissolved in acetic acid (50 mL) by warming on a water bath. To the hot solution was added pyridinium hydrobromide perbromide (0.8 g) and the reaction mixture was kept at room temperature for 1 h when yellowish crystals separated. These were filtered off, washed with water, and recrystallized from carbon tetrachloride to afford yellow needles of the dibromide **3g** (0.6 g, 43%), mp 172 °C (lit.[12] mp 150 °C).

Anal. Calcd for $C_{16}H_{12}Br_4O_3$: C, 33.6; H, 2.1. Found: C, 33.6; H, 1.9.

By the same procedure **3e** (0.64 g, 64%), **3f** (0.26 g, 47%), and **3h** (1.5 g, 57%) were obtained from **6a** (0.7 g), **6c** (0.4 g), and **6d** (1.8 g), respectively (see Table I).

Synthesis of 6,8-Dibromo-4'-methoxyflavone (4c). (i) 3',5'-Dibromo-2'-hydroxy-4-methoxychalcone (0.5 g) in amyl alcohol (15 mL) containing selenium dioxide (0.4 g) was refluxed at 140–150 °C for 12 h. The contents were filtered hot. The selenium was washed with a little ether. When the mother liquor was cooled, crystals of 6,8-dibromo-4'-methoxyflavone separated which were filtered off and recrystallized from ethanol to furnish white needles (0.3 g, 60%), mp 211 °C (lit.[13] mp 205 °C).

Similarly, **4e** (0.29 g, 65%), **4f** (0.31 g, 62%), and **4d** (0.31 g, 64%) were synthesized from **6a** (0.45 g), **6c** (0.5 g), **6d** (0.5 g), respectively.

(ii) The chalcone dibromide (0.5 g) was suspended in ethanol (10 mL) at room temperature and aqueous NaOH (10 M, 2 mL) was added. After 3 h the reaction mixture was acidified. The solid that separated was filtered off, washed with water, and crystallized from ethanol to give tiny white needles of 6,8-dibromo-4'-methoxyflavone (**4g**) (0.21 g, 59%) from ethanol, mp 210–211 °C. No depression in mixture melting point was observed with the previous product.

Under the same reaction conditions **3e**, **3f**, and **3h** (0.5 g of each) gave **4e** (0.21 g, 60%), **4d** (0.18 g, 53%), and **4f** (0.23 g, 65%), respectively.

Reaction of Flavone with Pyridine Perbromide. To flavone (0.3 g) in acetic acid (20 mL) at 40–60 °C was added pyridine perbromide (0.3 g) in small quantities. After 1 h the mixture was allowed to stand at room temperature for 5 days. It was then diluted with water to yield a white solid, which was filtered off and washed with water. Crystallization from ethanol gave 6-bromoflavone **4a** as white crystals (0.15 g, 37%), mp 190–191 °C. By the same procedure 4'-methoxy-6-methylflavone (0.3 g) gave **4d** (0.16 g, 41%), mp 207–208 °C. Co-TLC with an authentic sample[3] showed single spots with benzene–ethyl acetate (80:20).

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Registry No. 3a, 39729-11-8; **3b**, 43016-14-4; **3c**, 39729-17-4; **3d**, 22129-40-4; **3e**, 10372-55-1; **3f**, 75767-98-5; **3g**, 10372-59-5; **3h**, 29976-70-3; **4a**, 1218-80-0; **4b**, 75767-99-6; **4c**, 75780-70-0; **4d**, 29976-78-1; **4e**, 42079-81-2; **4f**, 75768-00-2; **5a**, 1214-47-7; **5b**, 3327-24-0; **5c**, 3033-96-3; **5d**, 16635-13-5; **6a**, 15482-67-4; **6b**, 75780-71-1; **6c**, 75768-01-3; **6d**, 29976-66-7; 4'-chloroflavone, 10420-75-4; flavone, 525-82-6; 4'-methoxy-6-methylflavone, 29976-77-0.

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Constituents of *Trichilia hispida* (Meliaceae). 3. Structures of the Cytotoxic Limonoids: Hispidins A, B, and C

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Limonoids, a group of highly oxidized triterpenoids, are known to occur in the Meliaceae family.[1-4] In this paper, the structure determinations of three limonoids of *Trichilia hispida* (Meliaceae), hispidins A (**1**), B (**2**), and C (**3**), whose isolation was previously described,[5] are reported.

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Table I. ^{1}H NMR Shifts (δ) and Coupling Constants (Hertz, in Parentheses) for Hispidins A (1), B (2), and C (3) and Hispidin A Acetate (1a) in $DCCl_3$

	1	2	3	1a
H-1	5.23 (dd, 13.1, 3.1)	7.56 (d, 12.3)[a]	7.57 (d, 12.3)[a]	5.19 (dd, 13.1, 3.1)
H-2	2.03 (dd, 13.1, 3.1)	6.09 (d, 12.3)	6.06 (d, 12.3)	2.02 (dd, 13.1, 3.1)
	2.58 (t, 13.1)			2.76 (t, 13.1)
H-5	2.98 (d, 9.5)	2.15 (dd, 9.6, 5.0)	2.15 (br)[a]	2.98 (d, 10.2)
H-6 β	2.75 (dd, 17.3, 9.5)	2.45 (dd, 15.1, 5.0)	2.48 (dd, 15.3, 4.9)	2.62 (dd, 17.0, 10.2)
H-6 α	1.68 (d, 17.3)	2.66 (dd, 15.1, 9.6)	2.66 (dd, 15.3, 9.6)	1.68 (d, 17.0)
H-9	4.16 (d, 8.1)	3.05 (d, 7.5)[a]	3.06 (d, 8.6)[a]	4.22 (d, 8.1)
H-11	5.25 (ddd, 11.2, 8.1, 0.7)	5.60 (ddd, 10.5, 7.5, 0.7)	5.58 (dd, 10.5, 8.6)	5.15 (ddd, 11.2, 8.1, 0.7)
H-12	6.05 (d, 11.2)	6.19 (d, 10.5)	6.18 (d, 10.5)	6.01 (d, 11.2)
H-15	5.60 (dd, 9.3, 5.1)	5.63 (dd, 9.6, 5.0)	5.60 (dd, 9.6, 5.0)	5.61 (dd, 9.8, 6.0)
H-16 α	2.13 (ddd, 14.5, 9.3, 5.1)	2.05 (ddd, 15.1, 9.8, 5.0)	2.03 (ddd, 14.7, 9.6, 5.0)	2.13 (ddd, 13.5, 9.0, 6.0)
H-16 β	2.42 (ddd, 14.5, 9.3, 9.3)	2.45 (ddd, 15.1, 9.8, 9.6)	2.42 (ddd, 14.7, 9.6, 9.6)	2.40 (ddd, 13.5, 9.0, 9.0)
H-17	3.96 (t, 9.3)	3.92 (t, 9.8)	3.92 (t, 9.6)	3.95 (dd, 9.8, 9.0)
H-18	1.00	1.02	1.00	1.00
H-19	1.39	1.13[a]	1.13[a]	1.39
H-21	7.16 (dd, 1.5, 0.7)	7.18 (dd, 1.5, 0.9)	7.18 (dd, 1.6, 0.7)	7.31 (dd, 1.8, 0.8)
H-22	6.28 (dd, 1.8, 0.7)	6.28 (dd, 1.7, 0.9)	6.28 (dd, 1.8, 0.7)	6.33 (dd, 1.7, 0.8)
H-23	7.33 (dd, 1.8, 1.5)	7.36 (dd, 1.7, 1.5)	7.37 (dd, 1.8, 1.6)	7.34 (dd, 1.8, 1.7)
H-28	1.30	1.69	1.69	1.34
H-29	3.61 (d, 8.5)	4.07 (d, 11.5)	4.06 (d, 11.9)	3.77 (d, 8.5)
	4.12 (d, 8.5)	4.26 (d, 11.5)	4.25 (d, 11.9)	4.10 (d, 8.5)
H-30(Z to C9)	5.31	5.18[a]	5.19[a]	5.28
H-30(E to C9)	5.85	5.25	5.23	5.81
H-2′	3.28 (br)[a]	3.17 (dd, 5.3, 2.9)	3.16 (dd, 5.5, 3.1)	4.66 (d, 4.2)
H-3′	1.52 (m)	1.51 (m)	1.50 (m)	1.60 (m)
2H-4′	1.15 (m)	1.12 (m)	1.13 (m)	1.20 (m)
H-5′	0.78 (t, 7.3)	0.80 (t, 7.2)	0.79 (t, 7.3)	0.78 (t, 7.3)
H-6′	0.86 (d, 6.8)	0.87 (d, 6.8)	0.86 (d, 6.8)	0.85 (d, 6.8)
H-3″	7.10 (qq, 7.1, 1.4)	6.82 (qq, 7.2, 1.7)		7.09 (qq, 7.0, 1.4)
H-4″	1.75 (qd, 7.1, 1.1)	1.82 (qd, 7.2, 1.1)		1.74 (qd, 7.0, 1.1)
H-5″	1.86 (p, 1.2)	1.82 (dq, 1.7, 1.1)		1.85 (p, 1.3)
OCHO	7.95 (d, 0.7)	7.93 (d, 0.7)	7.93	7.95 (d, 0.7)
OAc	2.02		2.12	2.03, 2.04, 2.11
COOMe	3.75			3.74
tertiary OH	4.00,[a] 4.21	2.71	2.90	4.12
secondary OH	2.69[a]	2.47 (d, 5.3)	2.50 (d, 5.5)	

[a] Broadened.

Their structures were deduced from spectral evidence, using comparisons with one another and with other limonoids from Meliaceae species. They will be discussed in order of increasing complexity, which is the reverse of the order in which they were eluted from a chromatography column (used to name them[5]).

Hispidin C, mp 245–246 °C, $[\alpha]^{25}_D$ −32°, was deduced from its spectra to have structure **3**, which had been assigned to a compound from *Aphanamixis polystacha* (Meliaceae) for which mp 239–242 °C and $[\alpha]^{25}_D$ −32° had been reported.[4] Direct comparison established the identity of these substances.[6] As the IR, UV, mass, and full ^{1}H NMR spectral data of this compound have not been reported, they are included herein.

Hispidin B (**2**) failed to crystallize, but was judged homogeneous from its TLC behavior and spectra. It displayed a molecular ion at m/e 712, which, combined with its elemental analysis, led to molecular formula $C_{38}H_{48}O_{13}$. In its UV spectrum, the position of its absorption maximum (214 nm) was essentially that of **3**, but the intensity (ϵ 22 180) was nearly twice that of **3**, indicating at least one extra chromophore. The IR spectrum of **2** was similar to that of **3** except for stronger bands at 1645 and 1260 cm^{-1}, suggesting a conjugated ester. The ^{1}H (Table I) and ^{13}C (Table II) NMR spectra of **2** showed all the peaks of **3**, except for those due to the acetate grouping, and showed extra peaks characteristic of tiglate esters; the stereoisomeric angelate ester is ruled out by the observed NMR shifts of H-3″ and H-4″.[7] Tiglate is also favored over angelate by the strength of the UV absorption (at λ_{217}, **2**

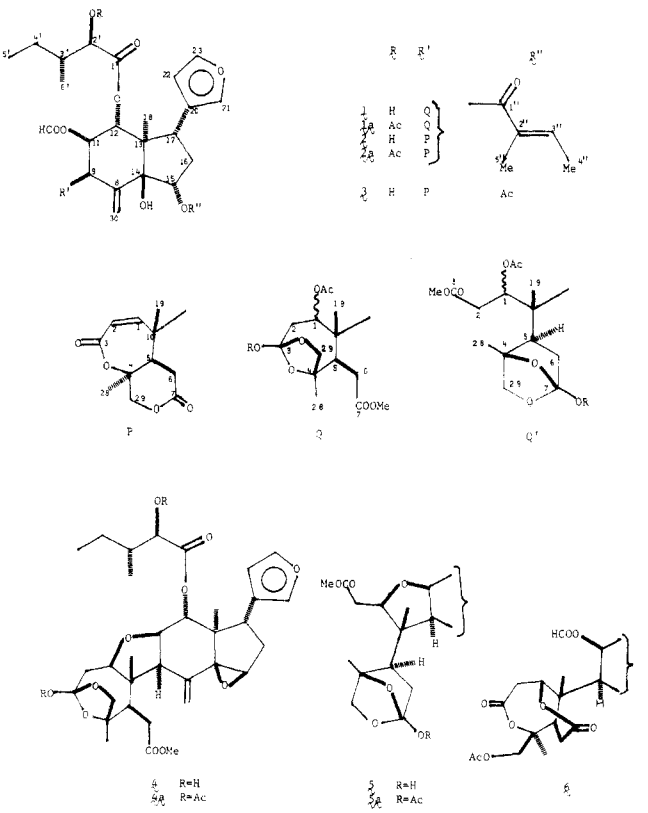

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Table II. ^{13}C NMR Chemical Shifts (δ) of Hispidins A (1), B (2), and C (3)

atom	1[a]	2[b]	3[a]
C-1	71.6 (d)	151.9 (d)	152.9 (d)
C-2	39.9 (t)	118.9 (d)	120.1 (d)
C-3	119.5 (s)	165.4 (s)	166.6 (s)
C-4	84.6 (s)	84.1 (s)	84.3 (s)
C-5	49.2 (d)	50.6 (d)	50.6 (d)
C-6	34.0 (t)	c	29.8 (t)
C-7	174.9 (s)	170.5 (s)	172.0 (s)
C-8	142.5 (s)	140.7 (s)	140.4 (s)
C-9	49.5 (d)	51.4 (d)	51.6 (d)
C-10	49.2 (s)	43.8 (s)	43.4 (s)
C-11	73.5 (d)	72.5 (d)	72.2 (d)
C-12	74.6 (d)	75.5 (d)	75.9 (d)
C-13	50.3 (s)	50.6 (s)	50.6 (s)
C-14	82.6 (s)	79.1 (s)	78.9 (s)
C-15	71.1 (d)	70.9 (d)	70.9 (d)
C-16	36.4 (t)	36.5 (t)	36.4 (t)
C-17	39.4 (d)	39.5 (d)	39.2 (d)
C-18	13.5 (q)	13.3 (q)	13.1 (q)
C-19	16.9 (q)	25.7 (q)	23.9 (q)
C-20	124.5 (s)	123.6 (s)	123.3 (s)
C-21	142.7 (d)	141.7 (d)	142.9 (d)
C-22	110.9 (d)	110.3 (d)	110.3 (d)
C-23	140.3 (d)	139.6 (d)	140.4 (d)
C-28	14.4 (q)	26.9 (q)	26.7 (q)
C-29	73.5 (t)	73.7 (t)	74.3 (t)
C-30	122.3 (t)	117.7 (t)	119.2 (t)
C-1′	174.9 (s)	172.6 (s)	174.8 (s)
C-2′	74.6 (d)	74.5 (d)	74.6 (d)
C-3′	38.3 (d)	37.6 (d)	37.5 (d)
C-4′	23.2 (t)	23.1 (t)	22.8 (t)
C-5′	11.6 (q)	11.1 (q)	11.1 (q)
C-6′	15.1 (q)	15.2 (q)	15.0 (q)
C-1″	167.6 (s)	165.4 (s)	
C-2″	128.1 (s)	127.1 (s)	
C-3″	138.8 (d)	137.4 (d)	
C-4″	28.4 (q)	27.4 (q)	
C-5″	22.8 (q)	23.3 (q)	
HC=O	161.2 (d)	159.7 (d)	159.6 (d)
MeC=O	169.9 (s)		169.4 (s)
MeC=O	21.1 (q)		20.6 (q)
MeO	52.7 (q)		

[a] In $DCCl_3$. [b] In CD_3COCD_3, due to low $DCCl_3$ solubility. [c] Obscured by solvent.

has ϵ 9000, greater than that of 3; tiglic acid has ϵ 10 700 and angelic acid has ϵ 5150 at this wavelength[8]). Hispidin B thus appeared to be 2, differing from hispidin C (3) only in being a 15α tiglate rather than a 15α acetate.

This view was strongly supported by the mass spectrum of 2. The molecular ion peak at m/e 712 and daughter ions at m/e 694 ($-H_2O$), 684 (-CO), 666 ($-H_2O$, CO), 648 ($-2H_2O$, CO), and 635 ($-CO_2$, H_2O, CH_3) were all accompanied by strong peaks at 100 mass units less (-tiglic acid). The presence of tiglate was further supported by very strong peaks at m/e 83 (tiglyl$^+$, base) and 55 (tiglyl$^+$ - CO). Acetylation of 2 gave **2a** with a molecular ion peak at m/e 754 and strong peaks at m/e 83 (base) and 55. Both 2 and **2a** had peaks at m/e 209 for grouping P (see structure). An alternative location of the tiglate grouping, at C-2′, was ruled out by prominent peaks due to the acetylated C-12 side chain in the spectrum of **2a** at m/e 157 ($C_8H_{13}O_3$, acylium ion), 129 (157 - CO), and 69 (129 - AcOH).

Hispidin A (1) also failed to crystallize but was judged homogeneous by TLC and its NMR spectra. Though neither 1 nor its acetate **1a** had a discernible molecular ion peak in the mass spectrum, the fragmentation peaks were readily interpretable in terms of molecular formulas $C_{41}H_{56}O_{16}$ for 1 and $C_{45}H_{60}O_{18}$ for **1a**, deduced with the aid of elemental analyses and carbon and hydrogen count by NMR.

All of the spectra pointed to hispidin A (1) differing from B (2) only in that grouping P was considerably changed; the other ring C and D substituents (including the tiglate at C-15) were the same. The NMR spectra (Table I and II) were especially helpful in showing this, as were strong mass spectral peaks at m/e 686 (M - tiglic acid and water) for 1 and m/e 770 (M - tiglic acid and water) for **1a**, m/e 157, 129, and 69 for **1a** (acetylated C-12 side chain), m/e 83 (tiglyl cation, base peak for both 1 and **1a**, composition verified by exact mass measurement) and 55 (tiglyl$^+$ - CO).

Group Q (R = H) was deduced for 1 from the following evidence. The CH_2CHOAc is required by the NMR spectra (see the H-1, H-2, and OAc peaks in Table I), as are a CO_2Me grouping, two methyls attached to quaternary carbons (one of which, from its ^{13}C NMR shift, is attached to oxygen), an additional CH_2CH grouping with no attachments to oxygen, a CH_2 in a five-membered ring (J_{gem} = 8.5 Hz) and between an oxygen and a quaternary carbon, and a hemiortho ester grouping (^{13}C NMR singlet at δ 119.5, tertiary OH in ^1H NMR). The two structures containing these features with a biogenesis suitably close to that of grouping P are Q and Q′, both of which fit the NMR data satisfactorily. Q and Q′ are analogous to groupings in 4 and 5, the two structures proposed for a substance formed by a treatment of dregeanin (6, from *Trichilia dregeana*[3]) with methanolic KOH;[9] the close relationship between this substance and 1 is indicated by its ^{13}C NMR peak for the carbon bearing three oxygens at δ 119.9, its H-29 geminal coupling constant of only 8 Hz, and its formation of a diaceate (**4a** or **5a**) without opening of a ring in the hemiortho ester (only slight changes in δ for H-29 hydrogens on acetylation, with J_{gem} remaining at 8 Hz).

Grouping Q for 1 is preferred over Q′ on biogenetic grounds, in that some six Meliaceae triterpenoids have been found with a methyl ester grouping located as in Q but none as in Q′.[2,4] Strong evidence favoring Q came from the mass spectra of 1 and **1a**, which lack peaks expected for Q′ (e.g., m/e 129 and loss of 129 for the bicyclic system in 1 and m/e 171 and loss of 171 in **1a**) but show the peaks expected for Q: m/e 301 for 1 and m/e 343 for **1a** (entire grouping Q$^+$), m/e 241 for 1 and m/e 283 and for **1a** (Q - AcOH), m/e 181 for 1 and m/e 223 for **1a** (Q - AcOH - HCOOMe), and m/e 167 for 1 and m/e 209 for **1a** (Q - AcOH - COOMe - Me). Because of the similarities in the spectra and acetylation behavior of 1 and the dregeanin degradation product 4 or 5, we favor structure 4 for the latter.

The question remains why 1 should prefer the cyclic hemiortho ester structure whereas the analogous 15-ketone (prieurianin[2]), 14β,15β-epoxide,[9] and even 15α-acetate ("substance 2"[4]) occur naturally in the hydroxy ϵ-lactone form. Possibly the larger 15α (tiglate) grouping in 1 tips the balance in favor of the cyclic structure, or the 1-acetate grouping in 1 may be β rather than α as in the other three compounds; this view is supported by 4 (1-oxygen β) existing as a hemiortho ester whereas the corresponding α compound does not.[9] ^1H NMR did not help here as the observed $J_{1,2\alpha}$ and $J_{1,2\beta}$ (3.1 and 13.1 Hz, the latter value indicating a dihedral angle close to 180°) fit the most stable appearing conformations of both the 1α and 1β acetates. The configurations of 1 at all chiral centers *except* C-1 are virtually certain from the X-ray study on priurianin 2′-*p*-bromobenzenesulfonate.[2] In any case, the absence of

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broadening of ^1H NMR peaks for groupings in the vicinity of the 9,10 bond in **1** and **1a**, in contrast to what is observed for prieurianin,[2] **2**, **3**, and many other compounds in this series suggests that steric congestion in this region has been alleviated in **1** and **1a** by cyclic hemiortho ester formation.

Experimental Section

^1H NMR spectra were run at 250 MHz on a Bruker WM-250 spectrometer. The assignments for hispidin A were checked by extensive decoupling. Other spectra were run as in earlier studies.[5] NMR parameters are given in Tables I and II.

Hispidin A (1) had UV [λ_{max} (EtOH) 213 nm (ϵ 12 000)], IR [($CHCl_3$) 3570 (OH), 3525 (OH), 3020 (C=CH), 1728 (ester), 1650 (C=C), 1380 (Me), 1210 (ester), 870 (furan) cm^{-1}], and mass (*m/e* 686, 635, 627, 626, 609, 594, 566, 527, 526, 509, 495, 484, 467, 449, 301, 241, 226, 209, 181, 167, 135, 83, 69, 55) spectra in accord with structure **1**.

Anal. Calcd for $C_{41}H_{56}O_{16}\cdot 2H_2O$: C, 58.57; H, 7.14. Found: C, 58.40; H, 7.00.

Hispidin A diacetate (1a), prepared from **1** with acetic anhydride and pyridine at 25 °C, had IR and mass (*m/e* 770, 728, 710, 682, 668, 640, 626, 611, 526, 512, 508, 343, 301, 283, 241, 226, 223, 209, 181, 167, 157, 135, 129, 83, 69, 55) spectra in accord with structure **1a**.

Anal. Calcd for $C_{45}H_{60}O_{18}\cdot 2H_2O$: C, 58.44; H, 6.93. Found: C, 58.55; H, 6.99.

Hispidin B (2) had IR [($CHCl_3$) 3590 (OH), 3010 (C=CH), 1760 (α,β-unsaturated lactone), 1725 (ester), 1645 (C=C), 1260 (ester), 1135 (*tert*-OH), 870 (furan) cm^{-1}] and mass spectra (see text) in accord with structure **2**.

Anal. Calcd for $C_{38}H_{48}O_{13}$: C, 64.04; H, 6.74. Found: C, 63.5; H, 7.1.

Hispidin B Acetate (2a). A small sample of **2** was treated with Ac_2O and pyridine at 25 °C; the mass spectrum of the resulting **2a** (amorphous) is described in the text.

Hispidin C (3), crystallized from methanol, had UV [λ_{max} (EtOH) 213 nm (ϵ 13 270)], IR [($CHCl_3$) 3590 (OH), 3020 (C=CH), 1760 (α,β-unsaturated lactone), 1730 (ester), 1645 (C=C), 1375 (Me), 1230 (ester), 1140 (*tert*-OH), 870 (furan), 775 (KBr, ethyl) cm^{-1}], and mass (*m/e* 672, 644, 626, 612, 595, 594, 586, 584, 566, 559, 548, 541, 513, 512, 481, 452, 435, 418, 354, 278, 243, 229, 225, 209) spectra in accord with structure **3**. Direct comparison (mixture melting point, chromatographic retention times, UV, and IR) with a sample of **3** from *Aphanamixis polystacha* established its identity.[6]

Hispidins A, B, and C demonstrated activities of $<1.0 \times 10^{-2}$, 2.9, and 17.0 μg/mL, respectively. Activity in the KB test system is defined as $ED_{50} \leq 20$ μg/mL.

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Registry No. 1, 75975-30-3; **1a**, 75975-31-4; **2**, 75975-32-5; **2a**, 75975-33-6; **3**, 70237-69-3.

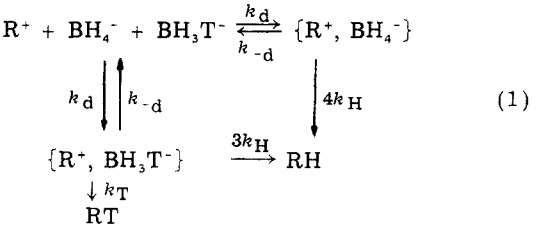
Communications

Tritium/Protium Discrimination in Reduction of Cyclopropenium Ion by Sodium Borohydride Does Not Identify the Rate-Determining Step[1]

Summary: Intramolecular isotope discrimination vitiates an attempt to deduce from the observation of a tritium isotope effect in borohydride reduction of a cyclopropenium ion that hydride transfer is at least partially rate determining.

Sir: It was recently reported[2] that the preparation of labeled sterculic acid, by dropping the corresponding cyclopropenium perchlorate into Me_2SO containing tritium-labeled sodium borohydride (26.7 mCi/mmol) at 5 °C, led to a product which had "incorporated 6.8 times less label than was present in the sodium borohydride". The product therefore presumably had a specific activity of 3.93 mCi/mmol. From this it was concluded that "the reaction is not solely diffusion controlled. The cation–hydride approach must be somewhat reversible with hydride transfer at least partially rate determining". The observed tritium discrimination is, however, consistent with either hydride transfer or diffusion as wholly rate determining, as we show below.

The two-step scheme incorporating diffusion and hydride transfer is shown in eq 1. Here it is assumed that the diffusion rate constants exhibit no isotope effect. Rate expressions for formation of RH and RT are given by eq 2, where secondary isotope effects have been neglected so



(1)

$$d[RH]/dt = 4k_H[\{R^+, BH_4^-\}] + 3k_H[\{R^+, BH_3T^-\}] \quad (2a)$$

$$d[RT]/dt = k_T[\{R^+, BH_3T^-\}] \quad (2b)$$

that k_H and k_T each refer to transfer of a specific hydrogen isotope from any species of borohydride ion. Equation 3

$$(d[RH]/d[RT]) = 4(k_H/k_T)([\{R^+, BH_4^-\}]/[\{R^+, BH_3T^-\}]) + 3(k_H/k_T) \quad (3)$$

is formed from the ratio of eq 2a to eq 2b. Now we take the ion pairs to be present at steady-state concentrations, yielding eq 4a; multiplication of numerator and denominator by $(0.25k_H)$ and definition of $\alpha = (k_{-d}/4k_H)$ produces eq 4b. The quantity α is useful because its value signifies

$$([\{R^+, BH_4^-\}]/[\{R^+, BH_3T^-\}]) = [(k_{-d} + 3k_H + k_T)/(k_{-d} + 4k_H)]([BH_4^-]/[BH_3T^-]) \quad (4a)$$

$$([\{R^+, BH_4^-\}]/[\{R^+, BH_3T^-\}]) = ([\alpha + 0.75 + (k_T/4k_H)]/[\alpha + 1])([BH_4^-]/[BH_3T^-]) \quad (4b)$$

the rate-determining step: as α approaches infinity, hydride transfer becomes completely rate determining, while as α approaches zero, diffusion becomes completely rate

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(2) Pawlowski, N. E.; Sinnhuber, R. O. *J. Org. Chem.* **1980**, *45*, 2735.

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