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JURIPIDINE, A 3-AMINO STEROIDAL ALKALOID FROM ROOTS OF *SOLANUM HISPIDUM**

AJIT K. CHAKRAVARTY, BINAYAK DAS and SATYESH C. PAKRASHI†

Indian Institute of Chemical Biology, Calcutta 700032, India

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Key Word Index—*Solanum hispidum*; Solanaceae; roots; steroidal alkaloids; (25*S*)-3β-amino-5α-spirostan-6α-ol; ^{1}H NMR; ^{13}C NMR.

Abstract—A new steroidal alkaloid designated as juripidine, together with jurubidine, has been isolated from the roots of *Solanum hispidum* and purified as its acetate. Its structure has been elucidated as (25*S*)-3β-amino-5α-spirostan-6α-ol by physical methods and by correlation with neochlorogenin.

INTRODUCTION

We earlier reported a number of new spirostane saponins and sapogenins from the leaves [1–3] and seeds [4] of *Solanum hispidum* wherein no alkaloid could be detected. We have now isolated a new 3-aminospirostane alkaloid, designated as juripidine (**1**), along with jurubidine (**2**), from the basic fraction of the methanol extract of the roots. This work is described in the present communication. The spirostane sapogenins, the major components of the leaves and seeds of *S. hispidum*, could not be found in the neutral fraction of the roots which furnished sitosterol and its β-D-glucoside. An analogous distribution of 3-aminospirostanes and nitrogen-free spirostanols was also found in S. *paniculatum* [5–7], from the roots of which jurubidine (**2**) was obtained for the first time by hydrolysis of the 3-aminofurostanol glucoside, jurubine [5], and in *S. torvum* [8].

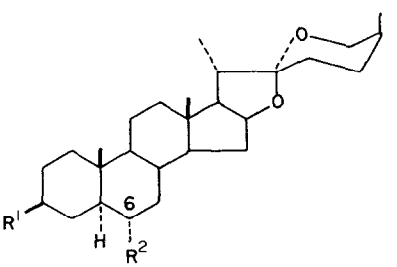
RESULTS AND DISCUSSION

The crude alkaloid mixture could only be purified through the corresponding acetates (Ac_2O–pyridine at room temperature) which when chromatographed over silica gel yielded two crystalline compounds, viz. *N*-acetyljurubidine (**3**), $C_{29}H_{47}NO_3$ and *N,O*-diacetyljuripidine (**4**), $C_{31}H_{49}NO_5$. *N*-Acetyljurubidine (**3**) was characterized by its conversion to neotigogenin acetate (**5**) on treatment with $NaNO_2$ in Ac_2O–HOAc [9] and the identity was established by direct comparison of **3** with an authentic specimen [6].

Although **3** could at least be partially hydrolysed on prolonged (18 hr) reflux with 40% KOH in MeOH–H_2O (4:1) to jurubidine (**2**), $C_{27}H_{45}NO_2$, **4** afforded only the *N*-monoacetate (**6**), $C_{29}H_{47}NO_4$, under the same conditions. The latter could not be hydrolysed further to juripidine (**1**) even under more drastic conditions (refluxing with 70% KOH for 36 hr). The structure of juripidine was therefore established through its diacetate (**4**).

N,O-Diacetyljuripidine (**4**) showed strong IR (KBr) absorption bands at 1733 and 1235 (OAc), and 1645 (NAc), besides the bands at 988, 967, 918, 895 and 850 cm^{-1} characteristic [10] of a spirostane ring system. This system was confirmed by the diagnostic [11] peaks at *m/z* 139 (base peak) and *m/z* 115 in its mass spectrum. It was also apparent from the mass spectral fragmentation that both the E- and F-rings of the spirostane skeleton were devoid of any additional nitrogen or oxygen substitution. The monoacetate (**6**) on oxidation with Kiliani's reagent gave a ketone (**7**). The hydroxyl group of **6** could thus be concluded to be secondary in nature.

The ^{1}H NMR spectrum (Table 1) of juripidine diacetate (**4**) exhibited a signal for the carbinyl proton at δ4.70 as doublets of a double-doublet (J = 12, 12 and 4 Hz) indicating the equatorial nature of the OAc group. The chemical shifts of 18-H_3, 21-H_3, 27-H_3, 26-H_2 and other assignable protons except 19-H_3 were very close (Table 1) to those of *N*-acetyljurubidine (**3**). The 19-H_3 signal of **4** and **6** exhibited a downfield shift by δ0.07 and



1 $R^1 = NH_2$, $R^2 = OH$ — **5** $R^1 = OAc$, $R^2 = H$

2 $R^1 = NH_2$, $R^2 = H$ — **6** $R^1 = NHAc$, $R^2 = OH$

3 $R^1 = NHAc$, $R^2 = H$ — **7** $R^1 = NHAc$, 6-keto

4 $R^1 = NHAc$, $R^2 = OAc$ — **8** $R^1 = OAc$, $R^2 = OAc$

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†To whom correspondence should be addressed.

Table 1. ^{1}H NMR chemical shifts* (δ) of the derivatives of alkaloids from *S. hispidum*

	19-H_3	18-H_3	21-H_3	27-H_3	26-H_2	16-H	3-H	6-H	NHAc	NAc	OAc
3	0.78	0.75	1.00 *d* (7)	1.05 *d* (7)	3.30 *d* (12), 3.96 *dd* (12, 3)	4.42 *m*	3.72 *m*	—	5.30 *d* (8)	1.92	—
4	0.85	0.75	1.00 *d* (7)	1.05 *d* (7)	3.30 *d* (12), 3.96 *dd* (12, 3)	4.40 *m*	3.70 *m*	4.70 *ddd* (12, 12, 4)	5.28 *d* (8)	1.93	2.02
6	0.80	0.74	0.99 *d* (7)	1.05 *d* (7)	3.30 *d* (12), 3.96 *dd* (12, 3)	4.40 *m*	3.72 *m*	3.72 *m*	5.32 *d* (8)	1.93	—
7	0.76	0.74	1.00 *d* (7)	1.07 *d* (7)	3.30 *d* (11), 3.94 *dd* (11, 3)	4.42 *m*	3.72 *m*	—	5.33 *d* (8)	1.94	—

*Spectra were recorded in $CDCl_3$ at 100 MHz. Values in parentheses are coupling constants in Hz.

0.02, respectively, compared to that of **3**. The above data were in excellent agreement [12–14] with the 6α-hydroxy-jurubidine structure for juripidine (**1**), supported by the ^{13}C NMR spectra (Table 2).

The almost identical ^{13}C chemical shifts for all the carbon atoms except C-4, C-19 and the B-ring carbons with those of **3** suggested that juripidine diacetate (**4**) differed from **3** only by an additional OAc substitution in ring B. The shielding of the signals for C-4 (5.8 ppm) and C-8 (1.4 ppm), and deshielding of C-5 (2.8 ppm) and C-7 (5.5 ppm) in the spectrum of **4** against those of **3** were in accordance with an additional equatorial OAc group at C-6 in **4**. The assigned structure was further corroborated by the virtually identical chemical shifts (Table 2) of the B-ring carbons of **4** with those of neochlorogenin diacetate (**8**) [15].

Finally, the structure of juripidine as (25*S*)-3β-amino-5α-spirostan-6α-ol (**1**) was confirmed by the conversion of **4** to neochlorogenin diacetate (**8**) with $NaNO_2$ in HOAc–Ac_2O (1:5) at 0° [9]. Isojuripidine, the (25*R*)-stereoisomer of **1**, has already been encountered in the roots of *S. paniculatum* by Italian workers [14, 16].

EXPERIMENTAL

Plant material. Roots of *S. hispidum* Pers., a prickly undershrub (~ 1.5–2 m) [17], were collected from Dehra Dun (India) by M/S. United Chemicals and Allied Products, Calcutta 700001. A voucher specimen (No. 1236) is available at the herbarium of the suppliers.

Extraction. Dried and powdered roots (1.5 kg) were defatted with petrol (60–80°) and then extracted with MeOH in a Soxhlet apparatus. The extract was concd, poured into 2 M HOAc with stirring and shaken with $CHCl_3$. Removal of organic solvent yielded a gum (30 g), which furnished only sitosterol and its β-D-glucoside on chromatography over silica gel. The HOAc-soluble part was basified with NH_3 soln and extracted with $CHCl_3$. Usual work-up afforded the total alkaloid (20 g) as a gum.

Acetylation of crude alkaloid mixture. The crude alkaloid was partially purified through chromatography over silica gel and the material eluted with petrol–$CHCl_3$ (4:1 to 1:4) was acetylated (Ac_2O–pyridine) at room temp. and the product chromatographed over silica gel using petrol–$CHCl_3$ (13:7) to obtain *N*-acetyljurubidine (**3**), mp 260–262° (from MeCN); $[\alpha]_D$ −78.12° ($CHCl_3$; *c* 0.32); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1666 (NAc), 988, 965, 917, 894, 846 (spirostane); MS *m/z* (rel. int.): 457 $[M]^+$ (15), 398 (6), 388 (8), 385 (8), 344 (12), 343 (34), 342 (11), 328 (7), 314 (13), 284 (3), 269 (3), 262 (10), 255 (9), 139 (100), 56 (8). Continued elution with petrol–$CHCl_3$ (1:3) afforded *N,O*-diacetyljuripidine (**4**), mp 264–266° (from MeCN); $[\alpha]_D$ −63.9° ($CHCl_3$; *c* 0.36); IR ν_{max}^{KBr} cm^{-1}: 1733, 1235 (OAc), 1645 (NAc), 988, 967, 918, 895, 850 (spirostane); MS *m/z* (rel. int.): 515 $[M]^+$ (5), 497 (2), 456 (12), 446 (3), 443 (7), 396 (6), 386 (8), 372 (6), 341 (15), 327 (8), 312 (9), 282 (12), 267 (6), 253 (23), 161 (23), 139 (100), 115 (5).

Table 2. ^{13}C NMR chemical shifts* of the acetates of alkaloids from *S. hispidum* and neochlorogenin diacetate (**8**)

Carbon No.	**3**	**4**	**8**†
1	37.2	37.4	36.8
2	28.5	28.4	27.1
3	48.9	49.2	73.0
4	35.3	29.5	28.4
5	45.3	48.1	48.5
6	28.8	72.1	72.0
7	32.1	37.6	37.8
8	35.1	33.7	33.7
9	54.3	53.5	53.6
10	35.5	36.5	36.6
11	20.9	20.7	20.9
12	40.0	39.7	39.8
13	40.5	40.4	40.5
14	56.2	55.8	55.9
15	31.7	31.5	31.6
16	80.8	80.6	80.6
17	62.0	61.9	61.9
18	16.5	16.3	16.4
19	12.3	13.2	13.3
20	42.1	42.1	42.1
21	14.3	14.2	14.3
22	109.6	109.5	109.6
23	27.0	27.0	27.1
24	25.8	25.7	25.7
25	26.0	25.9	25.9
26	65.1	65.0	65.1
27	16.0	16.0	16.0
OCO–Me	—	170.8	170.5
NCO–Me	169.2	169.0	—
OCO–CH_3	—	21.2	21.2; 21.4
NCO–CH_3	23.4	23.3	—

*Spectra were recorded in $CDCl_3$ and the chemical shifts are expressed on the δ scale with TMS as internal standard.

†Data incorporated from ref. [15].

Jurubidine (**2**) *from* N-*acetyljurubidine* (**3**). Hydrolysis of **3** (10 mg) with 40% KOH in MeOH–H_2O (4:1, 5 ml) under reflux for 18 hr gave jurubidine (**2**, 2 mg), mp 184–186° (from MeCN); $[\alpha]_D$ − 77.2° ($CHCl_3$; *c* 0.18); MS *m/z* (rel. int.): 415 $[M]^+$ (40), 400 (10), 385 (6), 356 (6), 346 (27), 343 (33), 329 (6), 326 (4), 301 (98), 286 (22), 284 (28), 272 (36), 269 (10), 255 (22), 245 (14), 139 (100), 115 (40), 82 (80), 69 (88), 56 (88).

N-*Acetyljuripidine* (**6**) *from* N,O-*diacetyljuripidine* (**4**). Hydrolysis of **4** (30 mg) under reflux with 5% KOH in MeOH–H_2O (4:1, 5 ml) for 30 min furnished **6** (25 mg), mp 284–286° (from MeCN); $[\alpha]_D$ − 54.2° ($CHCl_3$; *c* 0.24); IR ν_{max}^{KBr} cm^{-1}: 3210–3235(OH), 1637(NAc), 990, 965, 929, 900, 850 (spirostane); MS *m/z* (rel. int.): 473 $[M]^+$ (7), 458 (0.7), 456 (0.8), 414 (4), 404 (7), 402 (3), 401 (9), 360 (11), 359 (30), 343 (5), 330 (10), 327 (3), 282 (9), 267 (2), 253 (6), 139 (100), 115 (3), 69 (18), 60 (18), 56 (13).

Oxidation of **6**. To a soln of **6** (10 mg) in THF–Me_2CO (1:1, 5 ml), Kiliani's reagent (1.2 g CrO_3 in 10 ml 4 N H_2SO_4) was added dropwise with stirring at 0° until the colour persisted and then kept for 1 hr. Usual work-up yielded **7** (6 mg), mp 254–256° (from MeOH–MeCN); $[\alpha]_D$ − 48.3° ($CHCl_3$; *c* 0.29); IR ν_{max}^{KBr} cm^{-1}: 1710 (>C=O), 1645(NAc), 985, 918, 895, 850 (spirostane); MS *m/z* (rel. int.): 471 $[M]^+$ (8), 456 (2), 453 (3), 412 (9), 402 (15), 399 (9), 358 (23), 357 (65), 342 (14), 330 (18), 298 (20), 283 (7), 269 (5), 139 (100), 115 (12), 69 (16), 60 (12), 56 (10).

Neotigogenin acetate (**5**) *from* N-*acetyljurubidine* (**3**). To an ice-cold soln of **3** (20 mg) in HOAc–Ac_2O (1:5, 2 ml), $NaNO_2$ (50 mg) was added portionwise with stirring for a period of 3 hr and allowed to stand at 0° for a further 12 hr. Usual work-up gave neotigogenin acetate (**5**, 5 mg), mp 176° (lit. 179° [18]); ^1H NMR ($CDCl_3$): δ 0.76 (*s*, H_3-18), 0.83 (*s*, H_3-19), 0.98 (*d*, *J* = 7 Hz, H_3-21), 1.07 (*d*, *J* = 7 Hz, $H_{3,ax}$-27), 2.01 (*s*, OAc), 3.30 (*d*, *J* = 10 Hz, H-26), 3.96 (*dd*, *J* = 10, 3 Hz, H-26), 4.40 (*m*, H-16), 4.68 (*m*, H-3).

Neochlorogenin diacetate (**8**) *from* N,O-*diacetyljuripidine* (**4**). Compound **4** (20 mg) on treatment with $NaNO_2$ (50 mg) as above furnished neochlorogenin diacetate (**8**, 6 mg), mp 198–200° (lit. mp 200–202° [7]); IR ν_{max}^{KBr} cm^{-1}: 1732, 1245(OAc), 985, 918, 900, 860 (spirostane); MS *m/z* (rel. int.): 516 $[M]^+$ (6), 457 (4), 448 (8), 447 (4), 387 (5), 373 (5), 342 (8), 327 (18), 313 (5), 282 (24), 267 (5), 253 (9), 161 (23), 139 (100); ^1H NMR ($CDCl_3$): δ 0.76 (*s*, H_3-18), 0.90 (*s*, H_3-19), 0.98 (*d*, *J* = 7 Hz, H_3-21), 1.06 (*d*, *J* = 7 Hz, $H_{3,ax}$-27), 2.02, 2.03 (*s* × 2, OAc), 3.30 (*d*, *J* = 11 Hz, H-26), 3.95 (*dd*, *J* = 11, 3 Hz, H-26), 4.40 (*m*, H-16), 4.70 (*m* × 2, H-3, H-6).

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