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AZEDARACHOL, A STEROID ESTER ANTIFEEDANT FROM *MELIA AZEDARACH* VAR. *JAPONICA*

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Key Word Index—*Melia azedarach* var. *japonica*; Meliaceae; antifeedant; 2α,3α,16β-trihydroxy-5α-pregnane 20*R*-methacrylate.

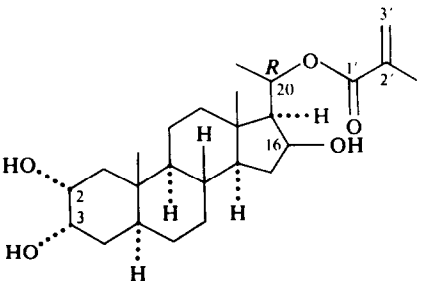
Abstract—A new steroid ester, azedarachol, from the root bark of *Melia azedarach* has been identified as an antifeedant against a Japanese insect pest and the structure has been assigned as 2α,3α,16β-trihydroxy-5α-pregnane 20*R*-methacrylate.

INTRODUCTION

In our investigation of insect antifeedants from the Meliaceae, we have isolated a number of limonoids from the African plant *Trichilia roka* [1–3]. *Melia azedarach* L. var. *japonica* Makino is a large tree found commonly in the southwest of Japan. From the fruits and stem bark, some bitter limonoids have been isolated [4–6]. We now describe a new steroid ester, azedarachol, isolated from the ether extract of the root bark, which showed antifeedant activity against the larvae of the insect pest *Ajrotis sejetum* Denis. with the leaf disk choice test (500 ppm). Some pregnane steroids have been found in Meliaceae and azedarachol is also a pregnane having the same *R* configuration at C-20 as the steroid hormone, 20β-dihydrogesterone, from *Khaya grandifoliola* [7].

RESULTS AND DISCUSSION

Azedarachol (**1**) was isolated in 0.003% yield from the ether extract of the dry bark by conventional CC and after recrystallization from methanol it exhibited the following spectral data; ν_{max}^{nujol} 3400 (OH), 1705 (conj. ester), 1603 (C=C), 1175, 1040 and 875 (term. methylene) cm^{-1}; λ_{max}^{MeOH} 213 nm (ε6800, conj. ester). It contained three secondary hydroxyls (δ3.84 *m*, 4.09 *br s* and 4.42 *m*) and yielded a triacetate (**2**) which on hydrolysis with 3% sodium carbonate afforded the monoacetate (**3**). On hydrogenation with palladium–carbon, **1** afforded a dihydro derivative (**4**) which did not show the IR band for the terminal methylene group nor the UV maxima for a conjugated system, and the carbonyl band was shifted to 1730 cm^{-1}. Furthermore, hydrolysis of **1** with 10% potassium carbonate afforded a tetraol (**5**); mp 280–282°. Azedarachol was characterized as a pregnane-type steroid carrying an ester side-chain. In the ^1H NMR spectrum, the C-18 and C-19 methyls appeared as two singlets at δ0.87 and 0.80, and the C-21 methyl as a doublet at δ1.37. There was a doublet of quartets at δ5.63 (H-20) coupling with the 21-methyl, leading to the inevitable conclusion that the ester group in the side chain is located at C-20.



1		**6**	2, 3—O_2CMe_2
2	2, 3, 16—OAc	**7**	2, 3, 16—OBz
3	16—OAc	**8**	16—OCOC(Me)=CH_2, 20—OH
4	20—$OCOCHMe_2$	**9**	2,3—O_2CMe_2, 16, 20—O_2CMe_2
5	20—OH		

Unequivocal support for the ring system and substitution in azedarachol was obtained from its EI mass spectrum in which there was a characteristic peak at *m/z* 249 (rel. int. 10%) denoting the tricyclic fragment commonly found in the spectra of pregnane steroids [8, 9] and two peaks of 231 (21%) and 213 (12%) indicated further successive losses of 1 and 2 mols of water suggesting the presence of two hydroxyl groups in rings A/B. Furthermore, there was a prominent peak at *m/z* 334 (15%), 86 amu loss than the $[M]^+$ ion (420 not observed) apparently due to the loss of CH_2=$CMeCO_2H$ which was also supported by the base peak at *m/z* 69 [CH_2=$CMeC^+$=O] [10, 11]. It contained two more strong peaks at 316 (68%) and 298 (51%) indicating further successive losses of 1 and 2 mol of water. The mass fragmentation of azedarachol is represented in Scheme 1. The presence of the methyl-propenoyl group was also deduced from the ^1H NMR spectrum in which there were an olefinic methyl at δ1.96 and a terminal methylene at δ6.16 and 5.52 showing allyl couplings with 1.4 Hz each other.

A ^1H NMR study of the acetate **3** at 400 MHz allowed

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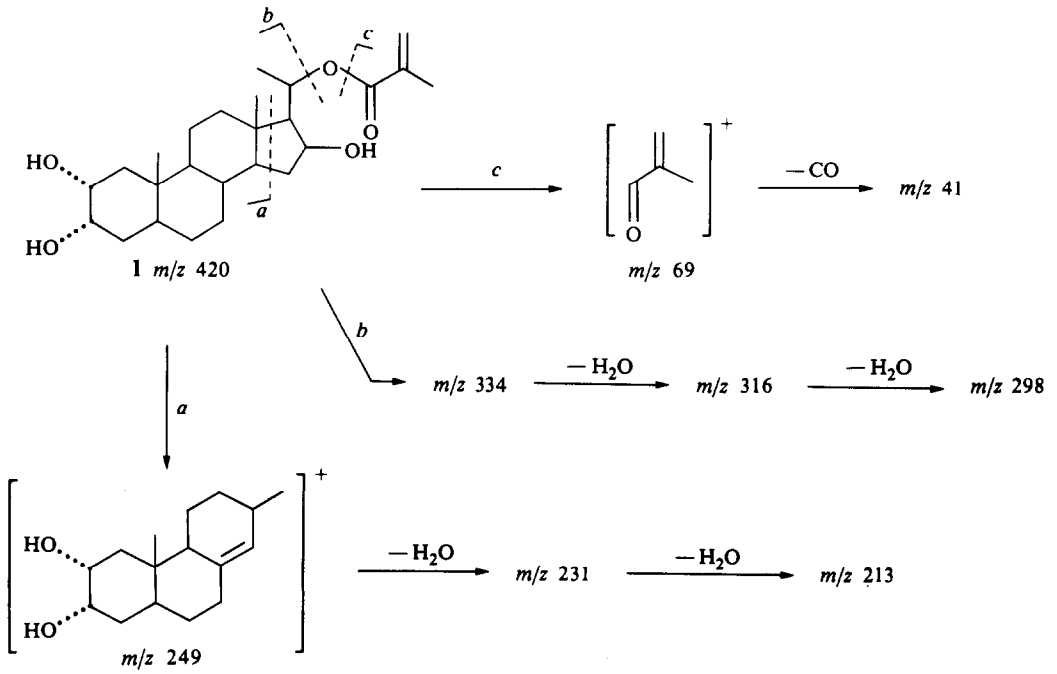


Scheme 1. Mass spectral fragmentation of azedarachol (1).

us to assign the peaks on the A and D rings as well as to derive structure 1 except for the A/B junction and the stereochemistry at C-20 (Table 1). The H-2β at δ4.96 coupling with the 1α- and 1β-H at δ1.39 (*dd*, J = 12.9 and 11.9 Hz) and δ1.70 (*dd*, J = 12.9 and 4.9 Hz), is coupled to the H-3β at δ5.27 with 3 Hz coupling with the H-4α and H-4β at δ1.66 and 1.43 with 3 Hz. On the other hand, the 16α-H at δ5.17 is coupled to H-15α and H-15β at δ1.25 and 2.44 with 4.1 and 7.8 Hz, and these protons are coupled to the H-14α at δ1.00, coupling with H-8β with 10.8 Hz, with 13.5 and 7.3 Hz, respectively. The H-16α, moreover, is coupled to H-17α at δ1.69 with 7.8 Hz, coupling with the H-20 at δ5.31 with 10.5 Hz [12].

The junction of rings A/B was clarified as *trans* from the

Table 1. ^{1}H NMR spectral data of **2** and **9** (400 MHz, $CDCl_3$, TMS as internal standard)

H	2	9
1α	1.39 *dd* (12.9, 11.9)	1.60 *dd* (11.9, 10.1)
1β	1.70 *dd* (12.9, 4.9)	1.92 *dd* (11.9, 7.0)
2	4.96 *ddd* (11.9, 4.9, 3.0)	4.11 *ddd* (10.1, 7.0, 4)
3	5.27 *q(br)* (3, 3, 3)	4.17 *td(br)* (4, 4, 2)
4α	1.83 *dt(br)* (13, 3)	1.85 *ddd* (15.3, 3.7, 2)
4β		1.61 *ddd* (15.3, 13.1, 4)
14	1.00 *ddd* (13.5, 10.8, 7.3)	0.87 *ddd* (13.1, 11.9, 7.7)
15α	1.25 *td* (13.5, 4.1)	1.27 *ddd* (13.5, 13.1, 3.4)
15β	2.44 *ddd* (13.5, 7.8, 7.3)	2.14 *dt* (13.5, 7.9)
16	5.17 *td* (7.8, 4.1)	4.40 *ddd* (7.9, 5.2, 3.4)
17	1.69 *dd* (10.5, 7.8)	0.94 *dd* (5.3, 5.2)
18	0.86 *s*	1.08 *s*
19	0.85 *s*	0.73 *s*
20	5.31 *dq* 10.5, 5.9)	4.35 *qd* (5.9, 5.3)
21	1.20 *d* (5.9)	1.34 *d* (5.9)
2'-Me	1.94 *t* (1.4)	—
3'	5.56 *dq* (1.6, 1.4)	—
	6.08 *dq* (1.6, 1.4)	—
O^{Me}	—	1.30 *s*, 1.44 *s*
O^{Me}	—	1.33 *s*, 1.49 *s*

Coupling constants (Hz) are in parentheses.

^{1}H NMR spectrum of an acetonide (**6**), in which H-4α at δ1.86 and H-4β at δ1.61 coupled to H-5 with 4.1 and 12.3 Hz, respectively, indicating its α, *axial*-orientation. Further confirmation of this was secured from a study of the CD spectrum of the benzoate **7**. It displayed a split CD with positive/negative Cotton effects at 237 nm (Δ_ε +8.7)/221 nm (Δ_ε −2.9) in methanol arising from the positively coupled oscillator.

The stereochemistry at C-20 was assigned *R* from the chemical shifts of the 13- and 20-methyls (Table 2). Irradiation of the 13-methyl of **2** induced 18% NOE on the H-20 signal which suggested the most stable conformation of the side chain, in which the 20β-oxygen was near to the 13-methyl group and its resonance frequencies should be subjected to great paramagnetic anisotropy by the C-20 oxygen and appeared at lower field. The reversal of this relationship in acylated compounds is due to the diamagnetic effect of the carbonyl function [13]. And so, the 13-methyl signal appeared at about δ0.86 in the spectra of the acylated compounds **1–3** and **6**, but it shifted to δ0.98 in a 20-hydroxyl compound (**8**); mp 206–207°; δ4.12 *m* (H-20) and 5.14 (*dq*, *J* = 4.1 and 7.9 Hz, H-16), obtained by the treatment of **4** with 4% potassium carbonate. A similar relationship was observed between the 20-methyl group and the 16β-oxygen. The 20-methyl signal showed at δ1.20 and 1.19 in the 16-acetylated compounds **2** and **3**. On the other hand, this signal appeared at δ1.37 and 1.36 in **1** and **4**. The stereochemistry of the 20-carbon was also revealed by the ^{1}H NMR spectrum of a diacetonide (**9**) (Table 1), in which the 13-methyl signal subjected to great anisotropy by the 20β, *axial*-oxygen, appeared at δ1.08 and the coupling constant of 5.3 Hz suggested the dihedral angle between H-17 and H-20 to be *ca* 17 or 150°. Dreiding model inspection of 9 revealed the angles to be *ca* 20 and 90° for the α- and β-orientations of the H-20 respectively.

All the above evidence leads to the structure of azedarachol as 2α,3α,16β-trihydroxy-5α-pregnane 20*R*-methacrylate (**1**).

EXPERIMENTAL

^{1}H NMR; 400 MHz, TMS as internal standard. Bioassay of the antifeedant was done by the leaf-disk method against three larvae of *Ajrotis sejetum* Denis.

Plant material. The root bark was collected in August 1981 at Kagoshima University, Kagoshima and identified by Dr. Sako (Kagoshima University).

Extraction and isolation. The dried root bark (860 g) was defatted with hexane and extracted with Et_2O to yield 12 g of an extract. The extract was chromatographed on silica gel with MeOH–CH_2Cl_2 and the crude compound was rechromatographed on silica gel with 10% Me_2CO–CH_2Cl_2 to give **1** (23 mg; 0.003% yield).

Table 2. ^{1}H NMR chemical shifts of methyl groups of **1** and its derivatives

Compound	H-18	H-19	H-21
1	0.87	0.80	1.37
2	0.86	0.85	1.20
3	0.84	0.80	1.19
6	0.86	0.73	1.36
8	0.98	0.73	1.17

Azedarachol (**1**). Crystallized from MeOH as colourless needles, mp 231–232°, $C_{25}H_{40}O_5$; FDMS *m/z*: 420 $[M]^+$; $[\alpha]_D^{24}$ +20.0 ($CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1705 (conj. ester), 1603 (C=C), 1175, 1040, 875 (term. methylene); UV λ_{max}^{MeOH} nm (log ε): 213 (3.83, conj. ester); EIMS *m/z* (rel. int.): 334 $[M-CH_2{=}CMeCO_2H]^+$ (15), 316 $[334-H_2O]^+$ (68), 298 $[316-H_2O]^+$ (51), 249 [rings A/B/C − H]$^+$ (10), 231 $[249-H_2O]^+$ (21), 213 $[231-H_2O]^+$ (12), 69 $[CH_2{=}CMeC{=}O]^+$ (100), 41 $[69-CO]^+$ (90); ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*, H-19), 0.87 (3H, *s*, H-18), 1.37 (3H, *d*, *J* = 5.9 Hz, H-21), 1.94 (3H, *t*, *J* = 1.4 Hz, H-2′), 2.31 (1H, *m*, H-15β), 3.76 (1H, *ddd*, *J* = 12.0, 4.8 and 3.0 Hz, H-2), 3.95 (1H, *br s*, H-3), 4.34 (1H, *dt*, *J* = 4.2 and 7.8 Hz, H-16), 5.34 (1H, *dq*, *J* = 10.5 and 5.9 Hz, H-20), 5.54 (1H, *dq*, *J* = 1.6 and 1.4 Hz, H-3′), 6.08 (1H, *dq*, *J* = 1.6 and 1.4 Hz, H-3′); ^{1}H NMR ($CDCl_3$ + 40%; pyridine-d_5): δ0.80 (3H, *s*, H-19), 0.91 (1H, *m*, H-14), 0.99 (3H, *s*, H-18), 1.38 (1H, *m*, H-15β), 1.42 (1H, *m*, H-1α), 1.47 (3H, *d*, *J* = 5.8 Hz, H-21), 1.51 (1H, *m*, H-4β), 1.58 (1H, *dd*, *J* = 10.5 and 7.6 Hz, H-17), 1.65 (1H, *m*, H-4α), 1.80 (1H, *dd*, *J* = 13.0 and 4.9 Hz, H-1β), 1.96 (3H, *br s*, 2′-Me), 2.27 (1H, *ddd*, *J* = 13.6, 7.8 and 7.4 Hz, H-15β), 3.84 (1H, *br d*, *J* = 12 Hz, H-2), 4.09 (1H, *br s*, H-3), 4.42 (1H, *m*, H-16), 4.84 (1H, *br s*, 2-OH), 5.26 (1H, *br s*, 3-OH), 5.52 (1H, *s*, H-3′), 5.62 (1H, *br s*, 16-OH), 5.63 (1H, *m*, H-20), 6.16 (1H, *s*, H-3′).

Triacetate. Acetylation of **1** (10 mg, Ac_2O–pyridine at room temp.) gave the triacetate **2** (12 mg), mp 160–161°; FDMS *m/z*: 547 $[M+1]^+$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1705, 1630, 1165, 875; UV λ_{max}^{MeOH} nm (log ε): 213 (3.65).

Monoacetate. MeOH (2 ml) soln of **2** (7 mg) was added to 3% Na_2CO_3 (2 ml) and stirred at room temp. for 2 hr. Work-up as usual gave **3** (3 mg), mp 241–243°; EIMS *m/z* (rel. int.): 462 $[M]^+$ (7), 444 $[M-H_2O]^+$ (4), 376 $[M-CH_2CMeCO_2H]^+$ (6), 316 $[376-AcOH]^+$ (71), 298 $[316-H_2O]^+$ (17), 69 $[CH_2{=}CMeC{=}O]^+$ (100); IR ν_{max}^{Nujol} cm^{-1}: 3400, 1730, 1700, 1630, 1170, 1060, 870; ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*, H-19), 0.84 (3H, *s*, H-18), 1.19 (3H, *d*, *J* = 5.8 Hz, H-21), 1.95 (3H, *br s*, 2′-Me), 2.03 (3H, *s*, OAc), 3.76 (1H, *m*, H-2), 3.96 (1H, *br s*, H-3), 5.16 (1H, *dt*, *J* = 4.1 and 7.8 Hz, H-16), 5.30 (1H, *dq*, *J* = 10.5 and 5.8 Hz, H-20), 5.57 (1H, *br s*, H-3′), 6.09 (1H, *br s*, H-3′).

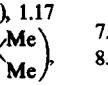
Dihydro derivative. Compound **1** (7 mg) was treated with Pd–C under H_2 gas in MeOH to give **4** (5 mg), mp 241–243°; FDMS *m/z*: 423 $[M+1]^+$; IR ν_{max}^{Nujol} cm^{-1}: 3400, 1730, 1160.

Hydrolysis of **1**. MeOH (2 ml) soln of **1** (7 mg) was added to 10% K_2CO_3 soln (2 ml) and refluxed for 3 hr. Work-up as usual gave **5** (5 mg), mp 280–282°; FDMS *m/z*: 353 $[M+1]^+$.

Acetonide. Compound **1** (5 mg) was stirred at room temp. for 3 hr in Me_2CO (3 ml) containing a small amount of 70% $HClO_4$. After making alkaline with $NaHCO_3$, the product was extracted and purified in the usual way to give **6** (3 mg), mp 234–235°; FDMS *m/z*: 460 $[M]^+$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1700, 1040, 920, 870; ^{1}H NMR ($CDCl_3$): δ0.73 (3H, *s*, H-19), 0.86 (3H, *s*, H-18), 1.33 and 1.49 (each 3H, ), 1.36 (3H, *d*, *J* = 5.8 Hz, H-21), 1.61 (1H, *ddd*, *J* = 15.2, 12.9 and 4.6 Hz, H-4β), 1.86 (1H, *ddd*, *J* = 15.2, 4.6 and 4.1 Hz, H-4α), 1.94 (3H, *dd*, *J* = 1.5 and 1.0 Hz, 2′-Me), 2.31 (1H, *dt*, *J* = 13.1 and 7.1 Hz, H-15β), 4.10 (1H, *ddd*, *J* = 11.3, 6.5 and 5.3 Hz, H-2), 4.18 (1H, *dt*, *J* = 5.3 and 4.6 Hz, H-3), 4.35 (1H, *m*, H-16), 5.34 (1H, *dq*, *J* = 11.0 and 5.8 Hz, H-20), 5.55 (1H, *sext*, *J* = 1.5 Hz, H-3′), 6.08 (1H, *dq*, *J* = 1.5 and 1.0 Hz, H-3′).

Benzoate. Benzoylation of **1** (5 mg, benzoyl chloride–pyridine at 60° for 24 hr) gave the tribenzoate **7** (5 mg); EIMS *m/z*: 732 $[M]^+$ (0.2), 646 $[M-68]^+$ (0.6), 610 $[M-PhCO_2H]^+$ (2), 524 $[646-PhCO_2H]^+$ (4), 488 $[610-PhCO_2H]^+$ (7), 402 $[524-PhCO_2H]^+$ (3), 280 $[402-PhCO_2H]^+$ (7), 105 $[PhCO]^+$

(100), 69 $[CH_2{=}CMeC{=}O]^+$ (3); UV λ_{max}^{MeOH} nm (log ε): 232 (4.26); CD (MeOH) nm: $\Delta\varepsilon_{221}$ −2.9, $\Delta\varepsilon_{237}$ +8.7 (π–π^* interaction bands); ^{1}H NMR ($CDCl_3$): δ1.00 (3H, *s*, H-19), 1.02 (3H, *s*, H-18), 1.24 (3H, *d*, *J* = 5.9 Hz, H-21), 1.95 (3H, *br s*, 2′-Me), 2.58 (1H, *ddd*, *J* = 13.4, 7.6 and 7.4 Hz, H-15β), 5.32 (1H, *ddd*, *J* = 11.3, 4.4 and 3.5 Hz, H-2), 5.42 (1H, *dt*, *J* = 4.0 and 7.6 Hz, H-16), 5.51 (1H, *dq*, *J* = 10.7 and 5.9 Hz, H-20), 5.57 (1H, *br s*, H-3′), 5.64 (1H, *br d*, *J* = 3 Hz, H-3), 6.10 (1H, *br s*, H-3′), 7.31 (2H, *br d*, *J* = 7.8 Hz), 7.45–7.52 (5H, *m*) 7.56–7.63 (2H, *m*), 7.86, 8.01 and 8.09 (each 2H, *br d*, *J* = 8 Hz).

Ester migration of **6**. To a 90% MeOH soln (2 ml) of **6** (3 mg), 4% K_2CO_3 soln (1 ml) was added and refluxed for 1.5 hr. Work-up as usual gave **8** (1.5 mg), mp 206–207°; FDMS *m/z*: 460 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ0.73 (3H, *s*, H-9), 0.98 (3H, *s*, H-18), 1.17 (3H, *d*, *J* = 6.1 Hz, H-21), 1.33 and 1.50 (each 3H, *s*, ), 1.91 (3H, *br s*, 2′-Me), 2.43 (1H, *ddd*, *J* = 13.4, 6.8 and 6.3 Hz, H-15β), 4.10 (1H, *m*, H-3), 4.12 (1H, *m*, H-20), 4.17 (1H, *m*, H-2), 5.14 (1H, *dt*, *J* = 4.1 and 7.9 Hz, H-16), 5.54 (1H, *sext*, *J* = 1.6 Hz, H-3′), 6.03 (1H, *br s*, H-3′).

Acetonide of **5**. Compound **5** (3 mg) was treated with a catalytic amount of 70% $HClO_4$ in Me_2CO at room temp. for 3 hr. Work-up as usual gave **9** (2.5 mg), mp 182–184°; FDMS *m/z*: 432 $[M]^+$.

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REFERENCES

1. Nakatani, M., James, J. C. and Nakanishi, K. (1981) *J. Am. Chem. Soc.* **103**, 1228.
2. Nakatani, M., Okamoto, M., Iwashita, T., Mizukawa, K., Naoki, H. and Hase, T. (1984) *Heterocycles* **22**, 2335.
3. Nakatani, M., Iwashita, T., Naoki, H. and Hase, T. (1985) *Phytochemistry* **24**, 195.
4. Ochi, M., Kotsuki, H., Hirotsu, K. and Tokoroyama, T. (1976) *Tetrahedron Letters* 2877.
5. Ochi, M., Kotsuki, H., Ishida, H. and Tokoroyama, T. (1978) *Chem. Letters* 99.
6. Ochi, M., Kotsuki, H., Ido, M., Nakai, H. and Tokoroyama, T. (1979) *Chem. Letters* 1137.
7. Adesogan, E. K. and Taylor, D. A. H. (1967) *Chem. Ind.* 1365.
8. Seifer, W. K., Gallegos, E. J. and Teeter, R. M. (1972) *J. Am. Chem. Soc.* **94**, 5880.
9. Takes, L., Jones, G., and Djerassi, C. (1968) *J. Am. Chem. Soc.* **90**, 5465.
10. Kupchan, S. M., Aynehchi, Y., Cassady, J. M., McPhail, A. T., Sim, G. A., Schnoes, H. K. and Burlingame, A. L. (1969) *J. Org. Chem.* **34**, 3867.
11. Kurokawa, T., Nakanishi, K., Wu, W. and Hsu, H. Y. (1970) *Tetrahedron Letters* 2863.
12. Chakravarty, A. K., Das, B. and Pakrashi, S. C. (1982) *Phytochemistry* **21**, 2083.
13. Lee, H. and Wolff, M. E. (1967) *J. Org. Chem.* **32**, 192.