Phytochemistry, Vol. 26, No. 6, pp. 1791–1795, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
© 1987 Pergamon Journals Ltd.

TRECHONOLIDE A, A NEW WITHANOLIDE TYPE FROM *TRECHONAETES LACINIATA*

DAVID LAVIE, ROBERTO BESSALLE, MAURICIO J. PESTCHANKER,* HUGO E. GOTTLIEB,† FELIX FROLOW and OSCAR S. GIORDANO*

Department of Organic Chemistry and Chemical Services, Weizmann Institute of Science, Rehovot 76100, Israel; *Department of Organic Chemistry, Universidad Nacional de San Luis, 786 General-Paz, 5700 San Luis, Argentina; †Department of Chemistry, Bar-Ilan University, Ramat Gan 52100, Israel

(*Received* 24 *July* 1986)

Key Word Index—*Trechonaetes laciniata*; Solanaceae; withanolides; steroidal lactones; trechonolide A; X-ray structure; trichloroacetyl isocyanate derivatives.

Abstract—The structure of a new withanolide type isolated from *Trechonaetes laciniata*, having a hemiacetal ring system and a 5-member ring lactone has been elucidated by X-ray analysis as (23*R*)-5β,6β-epoxy-12β,17β-dihydroxy-1-oxo-12,22-hemiacetal-ergosta-2,24-dien-23,26-olide (trechonolide A). A second compound (trechonolide B) having at C-12 a β-methoxy group, has also been isolated and assumed to be an artifact since, by heating trechonolide A in methanol with a trace of acid, the methoxy derivative was produced.

INTRODUCTION

The withanolides [1], a group of oxygenated steroidal lactones of the ergostane C-28 type, are known for the wealth of combinations encountered in their substitution patterns. These combinations enabled us to understand and provide a sequence for their biogenesis in the plant [2–4].

We wish to report two new types of withanolides of unusual structures isolated from the species *Trechonaetes laciniata* Miers collected in Argentina. The two compounds identified as trechonolide A and B have structures **1a** and **2a**, the former being determined by using X-ray single crystal analysis. The stereoscopic view of structure **1a** is shown in Fig. 1.

RESULTS AND DISCUSSION

Compound **1a**, $C_{28}H_{36}O_7$, shows in the UV spectrum an absorption at λ_{max} 218 nm (ε 17400) in ethanol. This value is characteristic for the overlapping of the two chromophores present in most withanolides, the α,β-unsaturated carbonyl in ring A and the unsaturated lactone system. The IR spectrum contains a signal at ν_{max} 1751 cm^{-1} which is characteristic for an unsaturated 5-member ring lactone.

The unusual characteristic feature of trechonolide A is a hemiacetal bridge formed from the 22-OH and C-12 ketone functions, resulting in a stable 6-membered ring with a β-oriented 12-OH group. Such a system, to the best of our knowledge, is unique in the withanolides group. Hemiacetals have been reported earlier between C-14 and C-13 or C-17 in the physalin group [5, 6]. At C-17 a β-oriented hydroxyl substituent is present which means that this compound belongs to the α-oriented side chain group of compounds [7]. Since the hemiacetal is connected to the C-22 carbon, a 5-membered ring lactone only could be formed. Such a γ-lactone system, although saturated, has been observed in ixocarpalactone A and B [8], and perulactones [9], however, both are in a β-

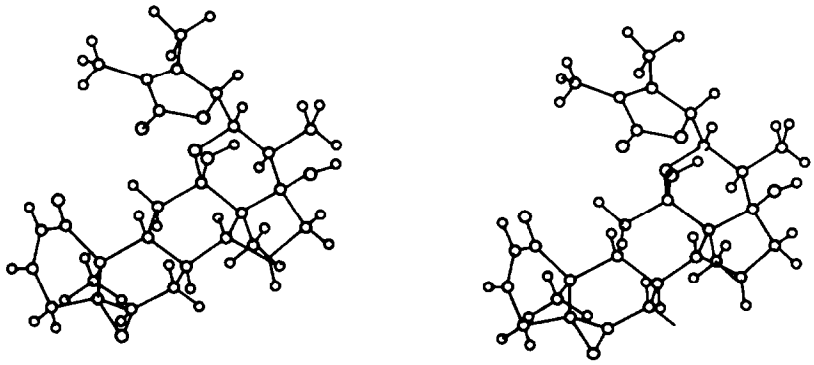
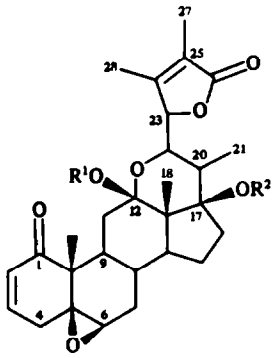
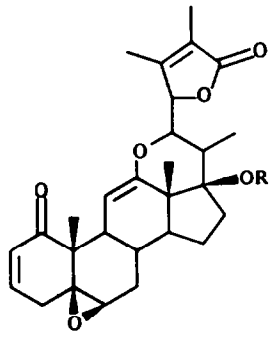


Fig. 1. Stereoscopic view of compound **1a**.





1a $R^1 = R^2 = H$
1b $R^1 = R^2 = TAC$
2a $R^1 = Me$, $R^2 = H$
2b $R^1 = Me$, $R^2 = TAC$

1c R = TAC

TAC = —CO.NH.CO.CCl_3

oriented side chain with hydroxyl groups attached at C-20 and C-22. In the present case the γ-lactone is unsaturated, being the first case reported for such a system. With this structure in hand the ^{1}H NMR spectrum was completely solved as given in Table 1. Dihedral angles from the X-ray diffraction data and the coupling constant values (J) for the most important protons are given in Table 2.

It can be seen that the ^{1}H NMR spectrum of **1a** shows the characteristic signals related to the 2-en-1-one system in ring A and a 5β,6β epoxide in ring B. The epoxidic H-6 has two vicinal protons at C-7 in a *gauche* relationship (Table 2) giving relatively small vicinal coupling constants (Table 1).

More interesting features are observed in rings D, E (for the hemiacetal) and the γ-lactone. A deshielding effect on H-20 induced by the 17-hydroxyl results in a signal at δ2.29. This signal appears as a sextuplet and was analysed by decoupling the signals related to Me-21 and to H-22 as being a doublet of $J = 11.2$ and a quartet of $J = 6.7$, respectively. The dihedral angle between H-20 and H-22 is shown in Table 2.

Trichloroacetyl isocyanate (TAI) is a well known reagent for determining hydroxyl groups in a molecule through the formation of the carbamate ester producing a singlet signal at low field (–OCONHCOCCl_3, –OTAC). The use of TAI derivatives on withanolides has been previously reported [11, 12]. Taking advantage of the X-ray analysis, and in order to understand the behaviour of this reagent in this new molecule, we carried out this analysis adding a few drops of TAI to the NMR tube containing **1a** dissolved in $CDCl_3$. The spectrum was monitored at predetermined time intervals. Since both hydroxyl groups in the molecule are β-oriented and free of any steric effects, they react completely within 6 minutes producing **1b** and resulting in the appearance of two signals at δ8.83 for 12-OTAC and δ8.71 for 17-OTAC. This assignment is based on the fact that at C-12 an adjacent oxygen may induce some additional deshielding effect thus producing the lower field signal. The signal corresponding to one of the H-11 appears now at δ3.42 (*dd*, $J = 14.0, 4.0$) due to the deshielding effect produced by the trichloroacetylcarbamate (OTAC) at the vicinal C-12. $J = 14.0$ is assigned to the geminal coupling and $J = 4.0$ to the vicinal coupling with 9α-H; the small J value is due to the dihedral angle (see Table 2). The signal of the 21-β Me is shifted by 0.18 ppm to lower field due to the proximity of the vicinal 17-OTAC which is also β and pointing in the same direction (Fig. 1).

In the spectrum taken 2 hr after reaction with TAI, we observed that the low field signals assigned to the two amidic NH were reduced in intensity by 50%, whereas a new peak appeared gradually in the same region at δ8.29. The magnitude of this signal was 50% of a proton, and this was an indication that one of the OTAC groups was undergoing elimination. This reaction was completed after 16 hr to form structure **1c** in which the two original low-field signals had completely disappeared, and the new signal had an intensity corresponding to a full proton. Furthermore, a new peak assigned to a vinylic H-11 at δ4.93 appeared; the double bond was produced by the elimination of the 12-OTAC. The possibility of the 17-OTAC being eliminated was discarded since a vinylic H-16, which is not part of conjugated ether system, should have appeared at a different location [3, 13]. An interesting observation was the fact that H-22 was found to be deshielded by 0.56 ppm which may be due, in part, to a charge distribution between the double bond and the ethereal oxygen. The fact that the new –NH– signal in **1c** is at higher field than the original two indicates that the two carbamates group were mutually deshielded, probably by hydrogen bonding between the –C=O and –NH– groups. With the complete elimination of the 12-OTAC, the singlet signal related to the 17-OTAC appeared now in its new position shifted by a sizable 0.42 ppm.

The second compound (**2a**) isolated was identified as the 12-methoxy derivative of **1a**. In the ^{1}H NMR spectrum of **2a** most of the signals are similar to those of **1a** with the addition of a sharp three-proton signal at δ3.20 assigned to a methoxy group. This was also confirmed by mass spectrometry, which showed a difference between the molecular ions of **1a** ($[M]^+$ 484) and **2a** ($[M]^+$ 498) of 14 mass units. Since the extraction procedure was carried out using boiling methanol for several hours, it seemed possible that the 12-OH of the hemiacetal was

Table 1. ^{1}H NMR spectral data for compounds **1a**, **1b**, **1c**, **2a** and **2b**

H	1a	1b	1c	2a	2b
2	5.98 *dd* (10.2; 2.8)*	6.00 *dd*	6.09 *dd*	5.99 *dd* (10.2; 3.0)	5.99 *dd*
3	6.82 *ddd* (10.2; 6.7; 2.3)	6.82 *ddd*	6.81 *ddd*	6.83 *ddd* (10.2; 6.3; 2.0)	6.84 *ddd*
4β	2.91 *dt* (*br*) (19.0; 2.6)	2.93 *dt* (*br*)	2.90 *dt* (*br*)	2.92 *dt* (*br*) (18.5; 2.5)	2.93 *dt* (*br*)
4α	1.87 *dd* (19.0; 6.7)				
6	3.10 *d* (*br*) (2.3)	3.13	3.16 *d* (*br*)	3.10 *d* (*br*) (2.3)	3.11 *d* (*br*)
7α	1.30 *ddd* (14.5; 11.2; 0.8)				
11α		3.42 *dd* (14.0; 4.0)	4.93 *d* (2.0)		
11β	1.69 *dd* (14.0; 12.6)				
12-OMe				3.20 *s*	3.42 *s*
12-OTAC		8.83 *s*			
14	1.20 *ddd* (12.3; 11.3; 4.5)				
17-OTAC		8.71 *s*	8.29 *s*		8.23 *s*
18-Me	1.00 *s*	1.10 *s*	1.13 *s*	0.95 *s*	0.94 *s*
19-Me	1.17 *s*	1.19 *s*	1.15 *s*	1.18 *s*	1.18 *s*
20	2.29 *dq* (11.2; 6.7)	2.62 *m*	2.08 *m*	2.28 *m* (*br*)	2.44 *m* (*br*)
21-Me	1.00 *d* (6.7)	1.18	1.16	0.98 *d* (7.6)	1.09 *d*
22	4.02 *dd* (11.2; 2.3)	3.99 *dd*	4.50 *dd*	3.73 *dd* (10.0; 1.3)	3.99 *dd*
23	4.80 *m* (*br*) ($W_{1/2}$ = 5.2)	4.87	4.82 *m* (*br*)	4.84 *m* (*br*)	4.86 *m* (*br*)
27-Me	1.82 *dq* (1.9; 1.0)	1.87 *s* (*br*)	1.90 *s* (*br*)	1.85 *s* (*br*)	1.86 *s* (*br*)
28-Me	1.94 *quintet* (1.0)	1.96 *s* (*br*)	2.08 *s* (*br*)	2.00 *s* (*br*)	2.04 *s* (*br*)

*Coupling constants (J) in Hz.

Table 2. Dihedral angles and coupling constant (J) of relevant protons of compound **1a**

Protons	Dihedral angle* (°)	J (Hz)
H_2–H_3	5.31	10.0
H_3–$H_{4\alpha}$	31.11	6.7
H_3–$H_{4\beta}$	−91.59	2.0
$H_{6\alpha}$–$H_{7\alpha}$	58.16	0.8
$H_{6\alpha}$–$H_{7\beta}$	−68.31	2.3
$H_{7\alpha}$–$H_{8\beta}$	−178.19	11.2
$H_{7\beta}$–$H_{8\beta}$	−53.30	
$H_{9\alpha}$–$H_{11\alpha}$	54.13	4.0
$H_{9\alpha}$–$H_{11\beta}$	176.56	12.6
$H_{8\beta}$–$H_{14\alpha}$	178.49	12.3
$H_{14\alpha}$–$H_{15\alpha}$	−157.44	11.3
$H_{14\alpha}$–$H_{15\beta}$	−29.41	4.5
$H_{20\alpha}$–$H_{22\beta}$	−176.42	12.0
$H_{22\beta}$–$H_{23\beta}$	62.09	3.0

*Dihedral angles are according to the right-hand rule [10].

methylated during the process, probably induced by traces of acid, and therefore trechonolide B was an artifact. In order to test this assumption, trechonolide A (**1a**) was heated to reflux in methanol with a drop of acetic acid. After 3 hours, **1a** was almost completely converted to trechonolide B (**2a**) as was seen in the ^{1}H NMR spectrum. Addition of TAI to the NMR tube afforded **2b**. The reaction was completed after 6 minutes. The signal corresponding to the 17-OTAC appeared at δ8.23, similar to **1c** (δ8.29). In addition, the shift of the Me-21 signal to δ1.09 further confirms the presence of OTAC at C-17 in **1c**.

The ^{13}C NMR data for trechonolide A (Table 3) are in agreement with the proposed structure. Thus, the chemical shifts for the A and B ring carbons are very similar to those reported for jaborosalactone L [14], which has the same 1-keto-Δ^2 and 5β,6β-epoxy functions as **1a**. The geminal oxysubstituents at C-12 cause shielding of C-9 and C-14 and deshielding of C-11, as expected. C-18 is very strongly deshielded, due to its position *gauche* to the equatorial 12-OH group. The signals for the sp^2 carbons in the γ-lactone ring are at lower field than the corresponding ones in the more usual δ-lactone [14, 15]. This is again in agreement with simpler models (compare, e.g. cyclo-

Table 3. ^{13}C NMR spectral data for compounds **1a** and **2a**

C	**1a**	**2a**
1	202.21	202.47
2	129.54	129.43
3	143.89	144.06
4	32.67	32.74
5	61.83	61.90
6	63.16	63.25
7	30.11	30.28*
8	29.31	29.39
9	41.88	41.86
10	47.35	47.39
11	36.40	30.33*
12	98.86	101.91
13	47.84	48.02
14	45.85	45.90
15	22.78	22.63
16	34.13	33.67
17	80.34	79.73
18	9.93	10.44
19	14.66	14.68
20	35.34	35.10
21	11.89	12.19
22	68.66	69.03
23	82.37	82.38
24	157.05	156.21
25	123.72	124.49
26	174.98	174.70
27	8.21	8.34
28	11.89	12.00
OMe	—	48.02

*Interchangeable.

pentenone to cyclohexenone [16]), and is of importance in recognizing the presence of a 5-member ring lactone.

Methylation of **1a** to **2a** results in deshielding of the dioxygenated C-12 (a β-effect) and shielding of C-11 (a γ-effect). The methoxy carbon is very shielded (δ48.02), as expected for an acetal.

Concerning the conformation of the ring system, a *cis* junction is observed for ring A/B. Ring A adopts a boat-like conformation with C-1 and C-4 above the plane. Ring B is a half chair with C-5, C-6, C-7 and C-10 nearly coplanar. Ring C is a normal chair and ring D adopts an envelope shape with C-13 above the plane. The hemiacetal ring E adopts the energetically stable chair conformation with C-13 now below the plane. The γ-lactone is attached α-equatorial to C-22 and is oriented away from the ring system, being itself planar. It is noteworthy that the conformation of the A, B, C and D rings is similar to that of withaferin A [17], while in withanolide E, ring D is also an envelope but with C-14 out of the plane [18].

Biogenetically one could assume that in the precursor, the α-oriented side chain should have two oxyfunctions at C-22 and C-23, the latter closing to form the 5-membered ring lactone, whereas, due to the α-orientation of the side chain, the former hydroxyl is in a good position to approach a C-12 carbonyl from the lower side of the molecule and form the hemiacetal bond.

The functional groups required to form the hemiacetal ring, namely, the 12-oxidized product (–C=O) [19], as well as the occurrence of a γ-lactone with a hydroxyl group in the side chain at C-22 [8, 9] have been reported. Also, the presence of a hydroxyl group at C-23 in a 6-member ring lactone in withanolide Q and R [20], support the biogenetic suggestion for the formation of a γ-lactone.

EXPERIMENTAL

Mps are (Fisher-Johns apparatus) uncorr; analytical TLC: silica gel F_{254}; ^1H and ^{13}C NMR: 270 and 300 MHz respectively, Fourier transform mode, $CDCl_3$, TMS as int. standard. Resolution-enhancement (Lorenzian to Gaussian conversion) was used to analyse the ^1H NMR spectrum of **1a**. Assignment of the carbon spectrum was aided by the techniques described in ref. [15]. X-ray single crystal analysis was made using 3-dimensional intensity data, collected on a computer controlled Enraf–Nonius (CAD-4 diffractometer) [λ (CuKα) = 1.5418 Å] by ω-2θ technique (θ < 77°) at room temp.

Isolation procedure. Air-dried powdered leaves of *Trechonaetes laciniata* Miers (2.5 kg), collected in Dec. 1984 from plants bearing flowers and fruits in the area of Laguna de los Horcones, Mendoza Argentina (classified by L. A. Del Vitto, IADIZA (CRICYT) Mendoza 5500, Argentina. Voucher specimen deposited at Herbario de la Universidad Nacional de San Luis no. 998) were extracted with hot MeOH (× 3). The solvent was evaporated under reduced pressure and the residues were extracted with *n*-hexane and Et_2O to give 45 and 38 g of residue respectively. The Et_2O extract was chromatographed on silica gel 60 H. Elution with C_6H_6–EtOAc (19:1) yielded compound **2a** (115 mg), and with C_6H_6–EtOAc (9:1) yielded **1a** (123 mg).

Trechonolide A (**1a**). Mp 264–266° from CH_2Cl_2–hexane; UV λ_{max}^{EtOH}: 218 nm (ε 17400); IR ν_{max}^{KBr} cm^{-1}: 3541, 3513, 3464, 3421, 3412, 1751, 1676, 1020, 1013, 994; MS (HR) *m/z* (rel. int.): 484.2436 $[M]^+$ (9.9), 466.2373 $[M-H_2O]^+$ (10.7), 448.2199 $[M-H_2O]^+$ (3.2), 373.2024 $[M-C_6H_7O_2]^+$ (100), 355.1944 $[M-C_6H_7O_2-H_2O]^+$ (53.1), 337.1742 $[M-C_6H_7O_2-2H_2O]^+$ (8.0), 315.1636 $[M-C_9H_{13}O_3]^+$ (77.5), 313.1393 $[M-C_9H_{15}O_3]^+$ (77.7), 297.1464 $[M-C_9H_{13}O_3-H_2O]^+$ (19.3), 279.1441 $[M-C_9H_{13}O_3-2H_2O]^+$ (11.3), 272.1461 $[C_{17}H_{20}O_3]^+$ (5.1), 269.1235 $[C_{17}H_{17}O_3]^+$ (34.3), 259.1340 $[C_{16}H_{19}O_3]^+$ (7.3), 253.1212 $[C_{17}H_{17}O_2]^+$ (15.1), 241.1241 $[C_{16}H_{19}O_3-H_2O]^+$ (8.9), 173.0972 $[C_{12}H_{13}O]^+$ (18.2), 161.0939 $[C_{11}H_{13}O]^+$ (19.1), 151.0762 $[C_9H_{11}O_2]^+$ (36.5), 141.0540 $[C_7H_9O_3]^+$ (15.8), 111.0464 $[C_6H_7O_2]^+$ (cleavage of C-20 to C-22), (21.9), 109.0650 $[C_7H_9O]^+$ (43.0), *m/z* $[M]^+$ 484.2436, $C_{28}H_{36}O_7$ requires: 484.2451.

Trechonolide B. 17β-hydroxy-12β-methoxy-5β,6β-epoxy-1-oxo-12,22-oxide-23R-ergosta-2,24-dien-23,26-olide (**2a**). CIMS isobutane reagent gas, *m/z* (rel. int.): 499 $[MH]^+$ (0.25), 481 $[MH—H_2O]^+$ (2.28), 467 $[MH—MeO]^+$ (31.87), 313 (100), 269 (32.38), 149 (26.29).

Conversion of **1a** *to* **2a**. **1a** (15 mg) was dissolved in 8 ml MeOH containing a drop of AcOH. The mixture was heated under reflux for 3 hr, the solvent evapd, the residue redissolved in 0.2 ml CH_2Cl_2 and passed through a small chromatographic column packed with silica gel 60 H (Merck). The product was eluted with 20 ml CH_2Cl_2–MeOH (20:0.8) yielding 9 mg of a residue containing more than 95% of **2a**.

Crystal data. Orthorombic, a = 11.429 (1), b = 11.905 (1), c = 18.527 (1) Å space group $P2_12_12_1$, and Z = 4. The structure was solved by direct methods and refined using 2794 unique reflections with $F_0 > 3\sigma(F_0)$. The non-hydrogen atoms were treated with anisotropic temp. factors. All hydrogens were found from a difference Fourier map and refined with isotropic temp. factors. Block-matrix least-squares refinement converged to R

= 0.036. The final difference Fourier map revealed only randomly distributed electron density (maximum peak of 0.20 eÅ^{-3}). Crystallographic data are available as supplementary material.

Acknowledgement—Thanks are due to Dr. M. Cojocaru, Bar-Ilan University for mass spectrometry measurements.

REFERENCES

1. Glotter, E., Kirson, I., Lavie, D. and Abraham, A. (1978) in *Bioorganic Chemistry* (van Tamelen, E. E., ed.) Vol. 2, p. 57. Academic Press, New York.
2. Kirson, I., Abraham, A. and Lavie, D. (1977) *Israel J. Chem.* **16**, 20.
3. Vande Velde, V. and Lavie, D. (1982) *Phytochemistry* **21**, 731.
4. Lavie, D. and Vande Velde, V. (1981) *Phytochemistry* **20**, 1359.
5. Glotter, E., Kirson, I., Abraham, A., Subramanian, S. S. and Sethi, P. D. (1975) *J. Chem. Soc. Perkin Trans.* 1, 1370.
6. Matsuura, T., Kawai, M., Nakashima, R. and Butsugan, Y. (1969) *Tetrahedron Letters* 1083.
7. Glotter, E., Abraham, A., Gunzberg, G. and Kirson, I. (1977) *J. Chem. Soc. Perkin Trans.* 1, 341.
8. Kirson, I., Cohen, A., Greenberg, M., Gottlieb, H. E., Glotter, E., Varenne, P. and Abraham, A. (1979) *J. Chem. Res.* S, 103; M, 1178.
9. Gottlieb, H. E., Kirson, I., Glotter, E., Ray, A. B., Sahai, M. and Ali, A. (1980) *J. Chem. Soc. Perkin Trans.* 1, 2700.
10. Klyne, W. and Prelog, V. (1960) *Experientia* **16**, 521.
11. Ramaiah, P. A., Lavie, D., Budhiraja, R. D., Sudhir, S. and Garg, K. N. (1984) *Phytochemistry* **23**, 143.
12. Kirson, I. and Gottlieb, H. E. (1980) *J. Chem. Res.* S, 338; M, 4275.
13. Gottlieb, H. E., Cojocaru, M., Sinha, S. C., Sahai, M., Bagchi, A., Ali, A. and Ray, A. B. (1986) *Phytochemistry* (in press).
14. Lavie, D., Gottlieb, H. E., Pestchanker, M. and Giordano, O. S. (1986) *Phytochemistry* **25**, 1765.
15. Gottlieb, H. E. and Kirson, I. (1981) *Org. Magn. Reson.* **16**, 20.
16. Kalinowski, H. O., Berger, S. and Braun, S. (1984) *^{13}C-NMR Spektroskopie*. Georg Thieme Verlag, Stuttgart.
17. McPhail, A. T. and Sim, G. A. (1968) *J. Chem. Soc. B*, 962.
18. Lavie, D., Kirson, I., Glotter, E., Rabinovich, D. and Shakked, Z. (1972) *J. Chem. Soc. Chem. Commun.*, 877.
19. Kalla, A. K., Raina, M. L., Dhar, K. L., Qurishi, M. A. and Snatzke, G. (1979) *Phytochemistry* **18**, 637.
20. Kirson, I., Cohen, A. and Abraham, A. (1975) *J. Chem. Soc. Perkin Trans.* 1, 2136.