Phytochemistry, Vol. 20, No. 4, pp. 803–806, 1981.
Printed in Great Britain.

0031-9422/81/040803-04 $02.00/0

NEW PRENYLATED ISOFLAVONES FROM *MILLETTIA PACHYCARPA**

ASHOK K. SINGHAL,† RAM P. SHARMA,† K. P. MADHUSUDANAN,† GOPALAKRISHNA THYAGARAJAN,† WERNER HERZ‡ and SERENGOLAM V. GOVINDAN‡

†Department of Organic Chemistry, Regional Research Laboratory, Jorhat-785 006, Assam, India; ‡Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.

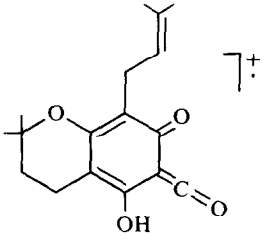
(*Received* 12 *May* 1980)

Key Word Index—*Millettia pachycarpa*; Leguminosae; Lotodoideae; prenylated isoflavones.

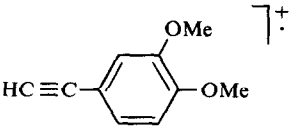
Abstract—Isolation of four new isoflavones from a new collection of *Millettia pachycarpa* is reported. Proof of the structure of lupinifolol, previously isolated from *M. pachycarpa*, by synthesis of dehydrolupinifolol is described.

INTRODUCTION

In an earlier article [1] we reported the isolation of the dihydroflavonol **1**§ and the isoflavones **2**, **3** and **4** from *Millettia pachycarpa* Benth. Conclusive proof for the structure of **1** has now been obtained by its dehydrogenation to **5a** which was in turn synthesized from kaempferol and 2-methylbut-3-en-2-ol via **6**. Investigation of *M. pachycarpa* from a different locality furnished not the previously isolated flavonoids, but the new isoflavones **7a**, **7b**, **10b** (or possibly **10a**) and **11a**.



A



B

RESULTS AND DISCUSSION

Compound **7a**, $C_{27}H_{32}O_7$, mp 140°, was a 5-hydroxyisoflavone (chelated 5-hydroxyl, H-2 at δ 7.95) with a 3,3-dimethyl-3-hydroxylpropyl group, a 3,3-dimethylchromane ring and two methoxyls distributed over the 2′, 5′, 6′, 6, 7 and 8-positions on the isoflavone skeleton (^{1}H NMR spectrum). The mass spectrum which *inter alia* gave rise to significant peaks at *m/e* 288 (**A**) and 162 (**B**) suggested the distribution indicated in the formula. That the two methoxyl groups were located at C-3′ and C-4′ was confirmed by oxidative hydrolysis to 3,4-dimethoxybenzoic acid; consequently the problem which remained was to decide whether the ring fusion was linear, as in **7a**, or angular. Dehydrogenation of the isoflavone with DDQ gave **8a**; the significant upfield shift in the signals of the vinylic hydrogens which accompanied acetylation to **8b** indicated that the pendant five carbon chain was attached to C-8 and not to C-6. Finally dehydration of **8b** to **9b** followed by hydrolysis gave a substance identical in all respects with authentic auriculasin 3′,4′-dimethyl ether (**9a**).

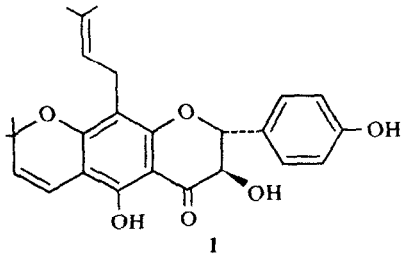
Isoflavone **7b**, mp 150°, was an isomer of **7a** with ring B substituted in the 2′, 4′, 3′, 4′ or 2′, 5′ positions (^{1}H NMR spectrum). That the chromane ring and the pendant 5-carbon side chain were attached to ring A was again suggested by the mass spectrum which in addition to peaks at *m/e* 288 and 162 also exhibited a peak at *m/e* 437 ($M^+ - 31$) indicative of methoxyl substitution on C-2′. Dehydrogenation of **7b** (DDQ) gave **8c** which on acetylation to **8d** exhibited the upfield shift of H-3″ and H-4″ characteristic of linear chroman ring fusion. Oxidative hydrolysis of **8d** gave 2,4-dimethoxybenzoic acid, thus permitting formulation of the parent isoflavone as **7b**.

A third new non-crystalline isoflavone, $C_{26}H_{28}O_6$, was characterized as a monoacetate and had two 3,3-dimethylchroman rings closed toward C-5 and C-7 of ring A as well as a methoxy group (^{1}H NMR spectrum). Ring B contained the hydroxyl at C-3′ or C-4′ (absence of a $M^+ - 17$ peak); if the former, the methoxyl was located at C-6′ and C-4′, if the latter, it was located at C-2′ or C-3′ (^{1}H NMR spectrum). Methylation followed by oxidative hydrolysis gave 3,4-dimethoxybenzoic acid; hence the parent compound was either **10a** or **10b**. A tentative decision in favor of **10b** was reached on the basis of the relatively large paramagnetic shift of H-5′, as compared with that of H-2′, on acetylation of the new flavone.

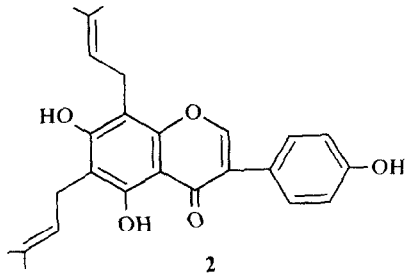
The most polar constituent was a monohydroxymonomethoxy isoflavone, mp 165°, which was isomeric with the preceding substance and had the same substitution pattern

* Work at the Florida State University supported in part by a grant (CA-13121) from the U.S. Public Health Service through the National Cancer Institute.

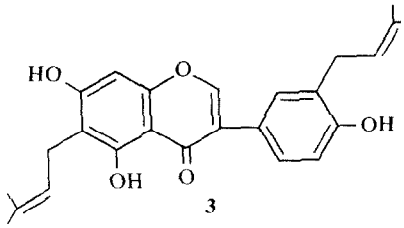
§In this article we overlooked prior isolation of **1**, named lupinifolol, by a group of Belgian workers [2].



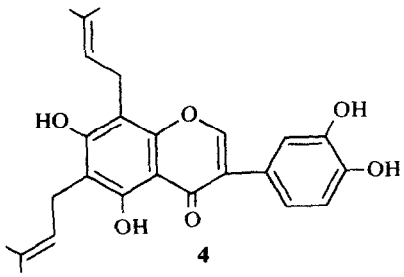
1



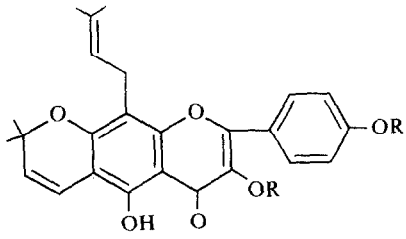
2



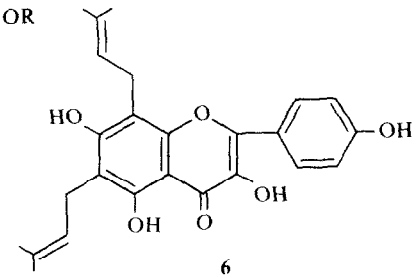
3



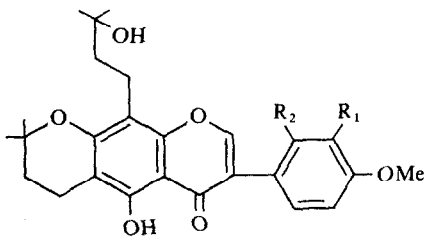
4



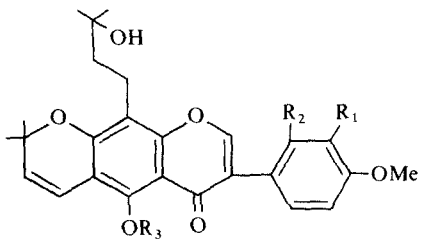
5a R = H
5b R = Me



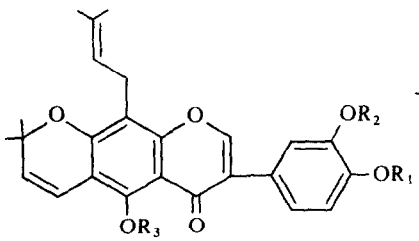
6



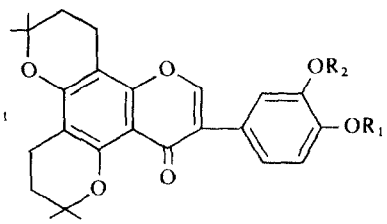
7a R_1 = OMe, R_2 = H
7b R_2 = OMe, R_1 = H



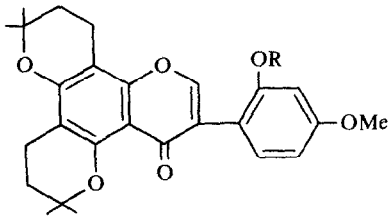
8a R_1 = OMe, R_2, R_3 = H
8b R_1 = OMe, R_2 = H, R_3 = Ac
8c R_1, R_3 = H, R_2 = OMe
8d R_1 = H, R_2 = OMe, R_3 = Ac



9a R_1, R_2 = Me, R_3 = H
9b R_1, R_2 = Me, R_3 = Ac
9c R_1, R_2, R_3 = H



10a R_1 = Me, R_2 = H
10b R_1 = H, R_2 = Me
10c R_1 = Me, R_2 = Ac
10d R_1 = Ac, R_2 = Me
10e R_1, R_2 = Me



11a R = H
11b R = Me

on ring A (^{1}H NMR spectrum). Methylation and oxidative hydrolysis gave 2,4-dimethoxybenzoic acid, hence the hydroxyl and methoxyl groups were attached to C-2′ and C-4′. As the mass spectrum of the parent compound exhibited a significant peak at *m/e* $M^+ - 17$ for which a peak at *m/e* $M^+ - 31$ was substituted in the MS of the methylated derivative, the hydroxyl group was located at C-2′ and the methoxyl at C-4′, as in **11a**.

EXPERIMENTAL

Dehydrogenation of **1**. A soln of 40 mg of **1** in 4 ml Me_2CO was refluxed with 100 mg K_2CO_3 until TLC indicated complete disappearance of starting material. The mixture was diluted with H_2O, acidified with HOAc and extracted with $CHCl_3$. The washed and dried extract was evapd and the residue purified by prep. TLC (C_6H_6–EtOAc, 6:1) to give 12 mg of **5a**, mp 82°; ^{1}H NMR ($CDCl_3$) 8.00 (*d*, *J* = 9 Hz, H-2′, H-6′), 6.84 (*d*, *J* = 9 Hz, H-3′, H-5′), 6.70 (*d*, *J* = 10 Hz, H-4″), 5.52 (*d*, *J* = 10 Hz, H-3″), 5.20 (*tbr*, *J* = 7 Hz, H-3‴), 3.45 (*d*, *J* = 7 Hz, H-2‴), 1.80, 1.68 (vinyl methyls), 1.45 (6H, two methyls); MS *m/e*: 420 (M^+), 405, 365, 311, 281 and 252. The material was converted to the methyl ether, mp 125°, lit. 122–123° [4].

Synthesis of **5a**. A soln of 95 mg of kaempferol in 1.5 ml dioxan was cooled to 0°, mixed with 6 drops of BF_3 etherate and then with 0.1 ml 2-methylbut-3-en-2-ol in 0.5 ml dioxan. After 2 hr at room temp. the mixture was poured into ice water and extracted with $CHCl_3$. The washed and dried extract was evapd and the residue purified by prep. TLC. The less polar material **6** was recrystallized from petrol, yield 9 mg, mp 150°, lit. 153–155° [4]; ^{1}H NMR ($CDCl_3$): 8.10 (*d*, *J* = 9 Hz, H-2′, 6′) 6.96 (*d*, *J* = 9 Hz, H-3′, H-5′), 5.20 (*t*, *J* = 7 Hz, two vinylic protons), 3.50 (4 benzylic protons), 1.86 (6 H) and 1.76 (6 H, methyls); MS *m/e*: 422 (M^+), 379, 367, 351, 323 and 311. The more polar material (45 mg) was kaempferol.

A soln of 9 mg of **6** and 5 mg of DDQ in 2 ml dry C_6H_6 was refluxed for 1 hr. The usual work up followed by prep. TLC (C_6H_6–EtOAc) gave **5a**, identical in all respects (TLC, ^{1}H NMR, mp, mmp) with **5a** from **1**.

Isolation of M. pachycarpa *constituents*. Air dried leaves (500 g) of *M. pachycarpa* Benth. collected in the Shella area, Shillong, Meghalya, on April 11, 1978, were extracted with $CHCl_3$ for 8 hr until the extract was colourless. The solvent was evapd at red. pres. and the residue (5 g) was dissolved in 200 ml MeOH and 20 ml H_2O and left overnight, filtered and the filtrate washed with petrol (60–80°, 5 × 150 ml). The MeOH portion was concentrated at red. pres. and extracted with $CHCl_3$ (4 × 150 ml). The $CHCl_3$ extracts were washed with H_2O, dried, evapd and the residue (1.10 g) was chromatographed over Si gel (200 g), 100 ml fractions were collected in the following order: 1–5, C_6H_6–EtOAc (9:1), 6–10, C_6H_6–EtOAc (4:1), 11–15, C_6H_6–EtOAc (2:1), 16–20, C_6H_6–EtOAc (1:1), 21–25, C_6H_6–EtOAc (1:2), 26–30, C_6H_6–EtOAc (1:4), 31–35, EtOAc.

TLC of fractions 4–9 showed two spots which were separated by prep. TLC (C_6H_6–EtOAc, 9:1). The compound (**7a**) with lower R_f obtained as a solid was recrystallized from petrol–EtOAc, mp 140°, yield 30 mg; UV λ_{max}^{MeOH} nm: 269; ^{1}H NMR (60 MHz, $CDCl_3$): δ 13.2 (5-OH), 7.95 (H-2), 7.25–7.00 (3 H, H-2′, H-5′, H-6′), 3.95 (two OMe), 2.80 (4 benzylic protons), 1.85 (4 homobenzylic protons), 1.43 (6 H) and 1.33 (6 H, methyls); MS *m/e*: 468 (M^+), 450, 435, 407, 395, 379, 351, 339, 325, 232, 295, 288, 233, 215, 177 and 162. (Calc. for $C_{27}H_{32}O_7$: MW, 468.2146. Found: MW (MS) 468.2138).

The compound (**7b**) with higher R_f was recrystallized from petrol, yield 33 mg, mp 150°; UV λ_{max}^{MeOH} nm: 271; ^{1}H NMR (60 MHz, $CDCl_3$): δ 13.0 (5-OH), 7.85 (H-2), 7.2–6.85 (H-3′, H-5′ and H-6′), 3.85 (two OMe), 2.70 (4 benzylic protons), 1.85 (4 homobenzylic protons), 1.50 (6 H) and 1.32 (6 H, methyls); MS *m/e*: 468 (M^+), 450, 437, 435, 419, 407, 395, 382, 379, 351, 345, 339, 327, 323, 295, 288, 233, 215 and 162. (Calc. for $C_{27}H_{32}O_7$: MW 468.2146. Found: MW (MS) 468.2128).

Fraction 10 showed one major spot and was purified by prep. TLC (C_6H_6–EtOAc, 6:1), yield 45 mg of **10a** (or **10b**) which could not be induced to crystallize; UV λ_{max}^{MeOH} nm: 268; ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.70 (H-2), 7.25 (*br*, H-2′), 6.86 (2 H, *br*, H-5′ and H-6′), 3.95 (OMe), 2.70 m (4 H), 1.80 m (4 H), 1.40 (12 H, methyls); MS *m/e*: 436 (M^+), 381 and 325. Acetylation (Ac_2O–pyridine) of 10 mg of this material gave 10 mg of **10c** (or **10d**), mp 212°: ^{1}H NMR ($CDCl_3$, 270 MHz): δ 7.79 (H-2), 7.33 (*br*, H-2′), 7.04 (*d*, *J* = 8.5 Hz, H-5′), 7.00 (*dd*, *J* = 8.5 Hz, 1.5, H-6′), 3.87 (OMe), 2.80 (*t*, *J* = 7 Hz, 2 H) and 2.64 (*t*, *J* = 7 Hz, 2 H), benzylic protons, 2.33 (acetate), 1.88 (*t*, *J* = 7 Hz, 2 H) and 1.83 (*t*, *J* = 7 Hz, 2 H, two methylenes), 1.41 (6 H) and 1.40 (6 H, methyls); MS *m/e*: 478 (M^+), 436 ($C_{26}H_{28}O_6$), 381 ($C_{22}H_{21}O_6$), 325 ($C_{18}H_{13}O_6$) and 149. (Calc. for $C_{28}H_{30}O_7$: MW, 478.1932. Found: MW (MS) 478.1957). Methylation of 15 mg of the isoflavone gave 15 mg of **10c** as a gum which was homogeneous by TLC, MS *m/e* at 450 (M^+). Hydrolysis with 30% H_2O_2 as described below for **7a** gave 4 mg of 3′,4′-dimethoxybenzoic acid, mp 179°.

Fraction 11 which showed a single spot on TLC was recrystallized from EtOAc–petrol, mp 165°, yield of **11a** 35 mg, UV λ_{max}^{MeOH} nm: 268; ^{1}H NMR ($CDCl_3$, 270 MHz): δ 7.80 (H-2), 7.32 (*d*, *J* = 1.5 Hz, H-3), 7.10 (*d*, *J* = 8 Hz, H-5′), 6.97 (*dd*, *J* = 8, 1.5 Hz, H-6′), 3.90 (OMe), 2.80 (*t*, *J* = 7 Hz, 2 H) and 2.63 (*t*, *J* = 7 Hz, 2 H, benzylic methylenes), 1.88 (*t*, *J* = 6.5 Hz, 2 H) and 1.83 (*t*, *J* = 6.5 Hz, 2 H methylenes), 1.43 (6 H) and 1.41 (6 H, methyls); MS *m/e*: 436 (M^+), 419 ($C_{26}H_{27}O_5$), 393 ($C_{23}H_{21}O_6$), 381 ($C_{22}H_{21}O_6$) and 325 ($C_{18}H_{13}O_6$). (Calc. for $C_{26}H_{28}O_6$: MW, 436.1826. Found: MW (MS), 436.1848). Methylation of 18 mg of **11a** with CH_2N_2 gave 18 mg of **11b** as a gum, ^{1}H NMR ($CDCl_3$): δ 7.80 (H-2), 6.8.7.2 (3 H of ring B), 3.80 (two OMe), 2.80 (2 H), 1.80 (4 H) and 1.40 (12 H); MS *m/e*: 450 (M^+), 419, 395 and 339. Hydrolysis of 12 mg of **11b** with alkaline H_2O as described below for **7a** gave 4 mg of 2,4-dimethoxybenzoic acid, mp 110°.

Reactions of **7a**. A soln of 20 mg of **7a** in 15 ml of dry C_6H_6 was refluxed with 15 mg of DDQ for 1 hr, concentrated at red. pres. and chromatographed over 50 g of Si gel. The column was eluted with C_6H_6–EtOAc (9:1), 50 ml fractions being collected. Fractions 3–5 showed a single spot on TLC and were combined to give **8a** as a gum (15 mg), ^{1}H NMR ($CDCl_3$): δ 13.2 (5-OH) 7.85 (H-2), 7.2–6.8 (H-2′, H-5′ and H-6′), 6.70 (*d*, *J* = 10 Hz, H-4″), 5.53 (*d*, *J* = 10 Hz, H-3″), 3.90 (two OMe), 2.70 (2 H, benzylic methylene), 1.85 (2 H, methylene), 1.53 (6 H) and 1.43 (6 H, methyls); MS *m/e*: 466 (M^+), 451, 448, 433, 419, 417, 405, 393, 379, 377, 375 and 351. Acetylation of 15 mg of **8a** (Ac_2O–pyridine) gave 15 mg of **8b** as a gum, ^{1}H NMR ($CDCl_3$): δ 7.80 (H-2), 7.2–6.8 (H-2′, H-5′ and H-6′), 6.42 (*d*, *J* = 10 Hz, H-4″), 5.60 (*d*, *J* = 10 Hz, H-3″), 3.85 (2 OMe), 2.70 (2 H, benzylic methylene), 2.40 (Ac), 1.90 (2 H, methylene), 1.50 (6 H) and 1.40 (6 H, methyls); MS *m/e*: 509 (M^+), 493, 466, 451, 448, 433, 405, 393, 379, 377, 375 and 339.

A soln of 5 mg of **8b** in 0.5 ml pyridine was mixed with 4 drops of $SOCl_2$ at 0° and allowed to stand for 15 min, diluted with ice cold water and extracted with $CHCl_3$. The washed and dried extract was evapd; the residue (**9b**) was dissolved in 0.5 ml of MeOH and 0.1 ml conc HCl, kept at room temp. for 8 hr, diluted with H_2O and extracted with $CHCl_3$. Removal of solvent gave **9a**, mp 98°, ^{1}H NMR ($CDCl_3$): δ 12.93 (5-OH), 7.80 (H-2), 7.20–6.70 (H-2′, H-5′ and H-6′), 6.60 (*d*, *J* = 10 Hz, H-4″), 5.40 (*d*, *J* = 10 Hz, H-3″), 5.00 (*t*, *J* = 7 Hz, vinyl proton of side chain), 3.80

(2 OMe), 2.80 (2 H, benzylic methylene), 1.70, 1.60, 1.40 (6 H, methyls); MS *m/e*: 448 (M^+), 419, 417, 405, 393, 375, 351. This substance was identical in all respects (TLC, ^{1}H NMR, MS) with auriculasin 3',4'-dimethyl ether synthesized by methylation of a sample of auriculasin (**9c**) [3, 5] with CH_2N_2.

To a soln of 10 mg of **7a** in 5 ml EtOH and 3 ml 15% aq. KOH was added, after stirring for 10 min, four 0.5 ml portions of 30% H_2O_2 were added over a 1.5 hr interval. The mixture was kept at room temp. for 4 hr, acidified with dil. H_2SO_4 and extracted with Et_2O. The Et_2O soln was extracted with 10% aq. $NaHCO_3$ (3 × 50 ml), the latter was acidified and extracted with Et_2O. Evapn of the solvent gave 2.5 mg of 3,4-dimethoxybenzoic acid, mp 178°, identical in all respects with an authentic sample.

Reactions of **7b**. A soln of 30 mg of **7b** in 20 ml dry C_6H_6 was dehydrogenated with 20 mg DDQ in the manner described for **7a**, the crude product being chromatographed over 50 g Si gel (eluant C_6H_6–EtOAc, 9:1, 15 × 50 ml fractions). Fractions 2–6 were combined to give 22 mg of **8c** as a gum, ^{1}H NMR ($CDCl_3$): δ 13.00 (5-OH), 7.85 (H-2), 7.2 6.85 (H-3', H-5' and H-6'), 6.70 (*d*, J = 10 Hz, H-4''), 5.55 (*d*, J = 10 Hz, H-3''), 3.90 (2 OMe), 2.70 (2 H, benzylic methylene), 2.00 (2 H, methylene), 1.57 (6 H) and 1.50 (6 H, methyls), MS *m/e*: 466 (M^+), 448, 433, 419, 417, 405, 393, 375 and 351. Acetylation of 10 mg of **8c** (Ac_2O–pyridine) gave 10 mg of **8d** as a gum, ^{1}H NMR ($CDCl_3$): δ 7.85 (H-2), 7.15–6.85 (H-3', H-5' and H-6'), 6.50 (*d*, J = 10 Hz, H-4''), 5.65 (*d*, J = 10 Hz, H-3''), 3.90 (2 OMe), 2.80 (benzylic methylene), 2.45 (Ac), 1.95 (2 H, methylene), 1.56 (6 H) and 1.50 (6 H, methyls); MS *m/e*: 508 (M^+), 466, 448, 433, 417, 405, 393, 377, 374 and 339.

Hydrolysis of 10 mg of **8c** with alkaline H_2O_2 as described for **7a** gave 2 mg of 2,4-dimethoxybenzoic acid, mp 108–110°, identical in all respects with an authentic sample.

Acknowledgements—We thank Dr. K. M. Shamsuddin, A. M. University, Aligarh, India, for a sample of auriculasin; Mr. R. C. Dass of our Institute for ^{1}H NMR spectra and Mr. L. C. Rabsha for identification of plant material.

REFERENCES

1. Singhal, A. K., Sharma, R. P., Thyagarajan, G., Herz, W. and Govindan, S. (1980) *Phytochemistry* **19**, 929.
2. Smalberger, T. M., Vleggaar, R. and Weber, J. C. (1974) *Tetrahedron* **30**, 3927.
3. Jain, A. C., Tuli, D. K. and Gupta, R. C. (1978) *J. Org. Chem.* **43**, 3446.
4. Jain, A. C. and Gupta, R. K. (1975) *Aust. J. Chem.* **28**, 607.
5. Minhaj, N., Khan, H., Kapoor, S. K. and Zaman, A. (1976) *Tetrahedron* **32**, 749.