7-OXO-7a-OXA-BRASSINOSTEROIDS WITH CHOLESTANE SIDE-CHAIN*

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New 7-oxo-7a-oxa analogs of brassinolide with cholestane side-chain were prepared. Analogs *VIII* and *IX* with 2,3-diol grouping exhibit a weak brassinolide activity. Of the synthesized compounds, the highest brassinolide activity was found for 3α,4α-dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*X*).

In the present communication we describe the synthesis of the so far unknown 7-oxo-7a-oxa analogs of the plant growth hormone brassinolide[1,2].

The synthesis (Scheme 1) starts from 3β-hydroxy-5α-cholestan-7-one[3] (*I*). Compound *I* was converted into tosylate *II* which on boiling in 2,4,6-collidine afforded a mixture of two isomeric olefins. The desired more polar olefin, which was the main reaction product, was identical[4,5] with the known 2(3)-olefin *III*; consequently, the more lipophilic olefin was the 3(4)-isomer *IV*. Olefin *III* was hydroxylated with osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide to give a mixture of two diols *V* and *VI* which were separated by chromatography. Their structure was determined by ^{1}H NMR spectroscopy. Comparison of chemical shifts of H-19 with the calculated values (Table I) has shown that the lipophilic diol is 2β,3β-dihydroxy-5α-cholestan-7-one (*VI*) whereas the polar diol is 2α,3α-dihydroxy-5α-cholestan-7-one (*V*). When treated with trifluoroperacetic acid in dichloromethane, compound *V* afforded only one product, although the reaction can give rise to two lactones: the 7-oxo-7a-oxa and 7-oxa-7a-oxo compounds. For the former structure, the ^{1}H NMR spectrum would exhibit one CH–O–C proton signal whereas in the spectrum the 7-oxa-7a-oxo lactone one would find two protons of the CH_2–O–C type. In our case the spectrum corresponded to the first alternative, showing one proton at 4.12 ppm as a doublet of doublets, and the product thus was 2α,3α-dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*VIII*).

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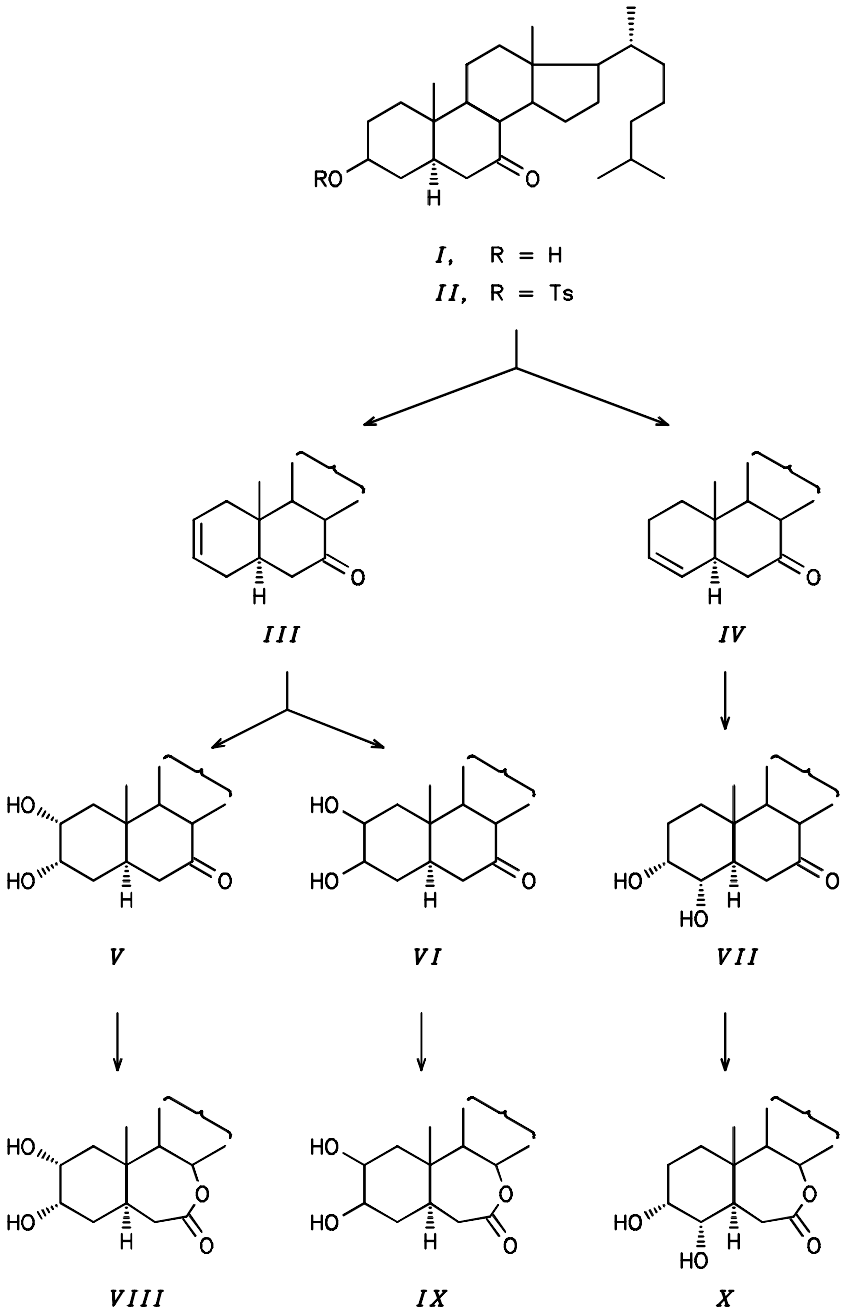

SCHEME 1

Also compound *VI* reacted with trifluoroperacetic acid under formation of a single lactone which was assigned the structure 2β,3β-dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*IX*) on the basis of reasoning analogous to that above.

The 3(4)-olefin *IV* was also hydroxylated with osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide to give diol *VII* as the sole product. In principle, both the 3α,4α- and 3β,4β-diols are possible, however, the former should be preferred for stereochemical reasons. Indeed, an analysis of ^{1}H NMR spectra confirmed this expectation (the calculated[6] position of the H-19 signal is 1.33 ppm for the 3β,4β-diol and 1.08 ppm for the 3α,4α-diol; the value experimentally found is 1.07 ppm). Similarly to diols *V* and *VI*, also the diol *VII* was subjected to Baeyer–Villiger reaction, affording 3α,4α-dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*X*).

An inspection of Dreiding models shows that the ring B in lactone *X* can exist in two interconvertible conformations (Fig. 1). In the ^{1}H NMR spectra, one of the H-6 protons has (in addition to the geminal interaction) a zero coupling constant with the H-5α proton, whereas for the second H-6 proton the coupling constant is high (J = 10.7 Hz). As seen from Newman projection (Fig. 2), this observation is compatible with both conformations: for the C(8)-chair form the coupling constants J(5α,6α) and J(5α,6β) should amount to 10.7 Hz and 0, respectively; for the C(7)-twist chair form the zero coupling constant should correspond to J(5α,6α) and the value of 10.7 Hz to J(5α,6β). Decisive in this respect was a NOE experiment which proved steric interaction of H-19 with the proton resonating at 2.55 ppm. This proton is 6β and axial and therefore the B-ring in compound *X* exists in conformation TC_7.

Thus, by the described procedures we obtained three analogs of brassinolide (compounds *VIII*, *IX* and *X*) and three analogs of castasterone (compounds *V*, *VI* and *VII*). According to preliminary bean second internode bioassays[7], all the compounds described exhibit a weak brassinolide activity. Surprisingly, compound *X* was the most potent.

TABLE I
Chemical shifts (ppm, δ-scale) of H-19 in ^{1}H NMR spectra of diols *V* and *VI*

Compound	H-19	
	Calculated[a]	Found
2α, 3α-Dihydroxy-5α-cholestan-7-one (*V*)	1.08	1.07
2β, 3β-Dihydroxy-5α-cholestan-7-one (*VI*)	1.35	1.28

[a] According to ref.[6].

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer PE 580 spectrometer in tetrachloromethane (unless stated otherwise), wavenumbers are given in cm^{-1}. Proton NMR spectra were taken in deuteriochloroform on a Varian XL-200 (FT mode, 200 MHz) instrument with tetramethylsilane as internal reference. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and multiplet half-widths ($W_{1/2}$) in Hz. The data were interpreted as the first-order spectra. Mass spectra were obtained with a ZAB-EG spectrometer at 70 eV. The identity of the samples prepared was checked by mixture melting points, thin-layer chromatography (TLC), IR and proton NMR spectra. Preparative TLC was carried out on 200 × 200 mm plates coated with 0.7 mm thick layer of silica gel Woelm DC. The "usual work-up" of a solution denotes washing with water, 5% aqueous potassium hydrogen carbonate, water, drying over sodium sulfate, filtering and evaporating the solvent to dryness in vacuo. The light petroleum used was a fraction boiling at 40 – 62 °C.

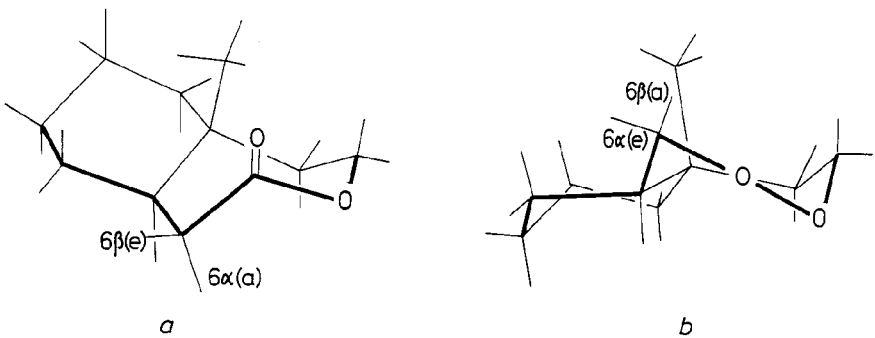

FIG. 1
Possible conformations of 3α,4α-dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*X*): *a* C(8)-chair, *b* C(7)-twist chair

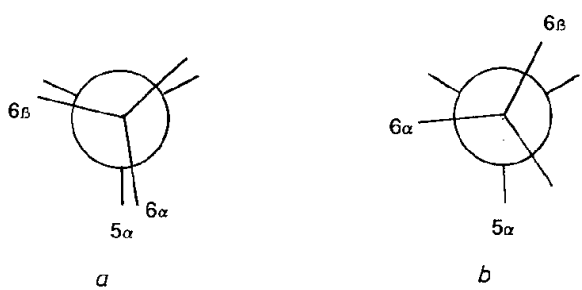

FIG. 2
Newman projection along the C(6)–C(5) bond in 3α,4α-dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*X*): *a* C(8)-chair, *b* C(7)-twist chair

3β-Tosyloxy-5α-cholestan-7-one (*II*)

p-Toluenesulfonyl chloride (5.0 g, 26.3 mmol) was added to a solution of 3β-hydroxy-5α-cholestan-7-one[4] (*I*; 5.0 g, 12.42 mmol) in pyridine (25 ml). After standing at room temperature overnight, the mixture was poured on ice, extracted with chloroform and worked up in the usual manner. Crystallization of the crude product from chloroform–ether afforded 2.1 g (56%) of tosylate *II*, m.p. 131 – 134 °C (decomposition). For $C_{34}H_{52}O_3S$ (540.9) calculated: 75.51% C, 9.69% H, 5.93% S; found: 75.12% C, 9.45% H, 5.50% S.

5α-Cholest-2-en-7-one (*III*)

The chromatography described in the preparation of olefin *IV* (vide infra) was continued by elution with benzene–light petroleum (1 : 4) and the eluted crude more polar olefin (3.7 g) was crystallized from methanol–acetone to give 1.6 g of product. Mother liquors on evaporation and crystallization from the same solvent mixture afforded further amount (0.7 g) of the product, raising the total yield to 2.3 g (48%). The product melted at 96 – 99 °C, in accord with the reported[5] value of 98 – 100 °C (an earlier reference[4] reported m.p. 156 – 158 °C). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 1.02 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6.5); 0.91 d, 3 H (3 × H-21, J = 6.5); 5.60 m, 2 H (H-2 and H-3, $W_{1/2}$ = 6). IR spectrum: 3 066, 3 026, 1 655 (C=C); 1 711 and 1 438 (CH_2C=O). For $C_{27}H_{44}O$ (384.6) calculated: 84.31% C, 11.53% H; found: 84.44% C, 11.49% H.

5α-Cholest-3-en-7-one (*IV*)

A solution of tosylate *II* (5.0 g, 8.98 mmol) in 2,4,6-collidine (20 ml) was heated at 150 °C for 8 h. After pouring into water, the reaction mixture was extracted with ether and worked up as usual. The obtained mixture of two olefins (shown by TLC to contain majority of the more polar *III*) was subjected to chromatography on silica gel (420 g) in light petroleum–benzene (2 : 1). Fractions containing the lipophilic olefin afforded 520 mg of residue which was crystallized from methanol to give 315 mg (9%) of crystalline olefin *IV*, m.p. 86 – 88 °C. Mass spectrum, *m*/*z*: 384 (M), 366 (M – H_2O). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 1.05 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6.5); 0.92 d, 3 H (3 × H-21, J = 6.5); 5.24 d, 1 H (H-4, J = 5); 5.64 dm, 1 H (H-3, J = 5). IR spectrum: 3 055, 3 022, 1 640, 1 652 sh (C=C); 1 711 and 1 432 (CH_2C=O). For $C_{27}H_{44}O$ (384.6) calculated: 84.31% C, 11.53% H; found: 84.37% C, 11.52% H.

2α,3α-Dihydroxy-5α-cholestan-7-one (*V*)

Osmium tetroxide (48 mg, 0.19 mmol) in 2-methyl-2-propanol (0.48 ml) was added to a solution of olefin *III* (960 mg, 2.5 mmol) in acetone (48 ml). After addition of *N*-methylmorpholine *N*-oxide (960 mg, 8.2 mmol), the reaction mixture was stirred in a nitrogen atmosphere at room temperature for 5 h and then left overnight. A solution of sodium sulfite (10%) was added, the mixture was stirred for 30 min and poured into water. The product was extracted with chloroform and worked up in the usual manner. The residue (910 mg) was crystallized from ethanol to give pure 2α,3α-diol *V* (330 mg, 32%), m.p. 217 – 219 °C. ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 1.07 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6); 0.91 d, 3 H (3 × H-21, J = 6.5); 3.80 m, 1 H (H-2β, $W_{1/2}$ = 22); 4.00 m, 1 H (H-3β, $W_{1/2}$ = 5). IR spectrum: 3 633, 3 577, 3 332, 1 037 (O–H); 1 710 (C=O). For $C_{27}H_{46}O_3$ (418.7) calculated: 77.46% C, 11.07% H; found: 77.37% C, 11.07% H.

2β,3β-Dihydroxy-5α-cholestan-7-one (*VI*)

Mother liquors from crystallization in the preceding experiment were evaporated to dryness and the residue was column chromatographed on silica gel in chloroform–2-propanol (199 : 1 to 33 : 1). Fractions containing the lipophilic compound afforded 86 mg (9%) of diol *VI*, m.p. 207 – 208 °C (ethanol). ^{1}H NMR spectrum: 0.64 s, 3 H (3 × H-18); 1.28 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6); 0.91 d, 3 H (3 × H-21, J = 6.5); 3.64 m, 1 H (H-3α, $W_{1/2}$ = 21); 4.04 d, 1 H (H-2α, J = 3, $W_{1/2}$ = 7.5). IR spectrum: 3 629, 3 577 (O–H); 1 711 (C=O). For $C_{27}H_{46}O_3$ (418.7) calculated: 77.46% C, 11.07% H; found: 77.37% C, 11.02% H.

3α,4α-Dihydroxy-5α-cholestan-7-one (*VII*)

Osmium tetroxide (158.4 mg, 0.83 mmol) and *N*-methylmorpholine *N*-oxide (3.15 g, 27.0 mmol) were added to a solution of olefin *IV* (3.15 g; 8.2 mmol) in acetone (157 ml) and the mixture was stirred at room temperature for 28 h. A solution of sodium sulfite (10%, 5 ml) was added, the mixture was stirred for 30 min and then poured into water. The product was taken up in chloroform, isolated as usual and the residue (3.25 g) was chromatographed on a silica gel column, using successively light petroleum–ether (1 : 1), light petroleum–ether–chloroform (1 : 2 : 1) and finally chloroform–ether (1 : 1) as eluents. Crystallization of the product (2.76 g) from ethanol afforded 1.1 g (40%) of diol *VII*, m.p. 228 – 230 °C. Mass spectrum, *m/z*: 418 (M), 400 (M – H_2O). ^{1}H NMR spectrum: 0.65 s, 3 H (3 × H-18); 1.07 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6); 0.91 d, 3 H (3 × H-21, J = 6.5); 2.1 – 2.7 m, 3 H (H-8 and 2 × H-6); 3.51 dd, 1 H (H-4β, J = 2.2; J' = 10.8); 3.98 dm, 1 H (H-3β, $W_{1/2}$ = 6.5). IR spectrum: 3 629, 3 621 sh, 3 564, 3 437, 1 070, 1 049, 1 030 (O–H); 1 702 (C=O). For $C_{27}H_{46}O_3$ (418.7) calculated: 77.46% C, 11.07% H; found: 77.37% C, 11.02% H.

2α,3α-Dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*VIII*)

A solution of trifluoroperacetic acid (freshly prepared from trifluoroperacetic anhydride (904 mg) and 50% hydrogen peroxide (0.147 ml) in dichloromethane (5 ml)) was added to a solution of diol *V* (209.3 mg, 0.50 mmol) in dichloromethane (3 ml). After standing at room temperature for 4 h, the reaction mixture was poured into water and the product was extracted with chloroform. The chloroform extract was washed with 10% sodium hydrogen carbonate and water, dried over sodium sulfate and the solvent was distilled off in vacuo. The residue was purified by chromatography on a column of silica gel (95 g) in chloroform–2-propanol (49 : 1). Crystallization of the obtained material (165 mg) from aqueous ethanol afforded 49 mg (26%) of lactone *VIII*, m.p. 212 – 214 °C. Mass spectrum, *m/z*: 434 (M), 416 (M – H_2O), 398 (416 – H_2O), 304 (M – H_2O – C_8H_{16}). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 0.99 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6); 0.91 d, 3 H (3 × H-21, J = 6); 3.64 m, 1 H (H-2β, $W_{1/2}$ = 21); 3.95 m, 1 H (H-3β, $W_{1/2}$ = 7.5); 4.12 dd, 1 H (H-8, J = 8; J' = 10). IR spectrum: 3 608, 3 567, 1 034, 1 019 (O–H); 1 720, 1 156, 1 034, 1 019 (lactone). For $C_{27}H_{46}O_4$ (434.7) calculated: 74.61% C, 10.67% H; found: 79.61% C, 10.85% H.

2β,3β-Dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*IX*)

A solution of trifluoroperacetic acid (freshly prepared from trifluoroperacetic anhydride (3.72 g) and 50% hydrogen peroxide (0.6 ml) in dichloromethane (20.6 ml)) was added to a solution of diol *VI* (0.86 g, 2.05 mmol) in dichloromethane (12.3 ml). After standing at room temperature for 20 h, the mixture was worked up as described in the preceding experiment. The obtained residue (1.2 g) was twice crystallized from aqueous ethanol; yield 0.45 g (51%) of lactone *IX*, m.p. 233 – 235 °C (ethanol).

Mass spectrum, *m/z*: 434 (M), 416 (M – H_2O), 398 (416 – H_2O). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 1.18 s, 3 H (3 × H-19); 0.86 d, 6 H (3 × H-26 and 3 × H-27, J = 6); 0.90 d, 3 H (3 × H-21, J = 6.5); 2.89 dd, 1 H (H-6, J = 10; J' = 14.6); 3.65 m, 1 H (H-3α, $W_{1/2}$ = 21); 3.99 d, 1 H (H-2α, J = 2); 4.18 dd, 1 H (H-8, J = 8.5; J' = 10). IR spectrum (chloroform): 3 620, 3 613 sh, 3 567, 3 435 (O–H); 1 719, 1 304, 1 153 (lactone). For $C_{27}H_{46}O_4$ (434.7) calculated: 74.61% C, 10.67% H; found: 74.30% C, 10.15% H.

3α,4α-Dihydroxy-7a-oxa-B-homo-5α-cholestan-7-one (*X*)

A solution of trifluoroperacetic acid (freshly prepared from trifluoroperacetic anhydride (2.16 g) and 50% hydrogen peroxide (0.35 ml) in dichloromethane (12 ml)) was added to a solution of diol *VII* (0.50 g, 1.19 mmol) in dichloromethane (7.15 ml). After standing at room temperature for 6 h, the mixture was worked up as described in the preceding experiment. The residue (520 mg) was twice crystallized from anhydrous ethanol; yield 169 mg (33%) of lactone *X*, m.p. 250 – 252 °C. Mass spectrum, *m/z*: 434 (M), 416 (M – H_2O), 398 (416 – H_2O). ^{1}H NMR spectrum: 0.67 s, 3 H (3 × H-18); 0.99 s, 3 H (3 × H-19); 0.861 d, 3 H and 0.864 d, 3 H (3 × H-26 and 3 × H-27, J = 6.5); 2.08 – 3.00 m, 2 H (2 × H-6); 3.37 dd, 1 H (H-4β, J = 11; J' = 2.5), 4.01 m, 1 H (H-3β, $W_{1/2}$ = 7.5); 4.24 dd, 1 H (H-8, J = 9; J' = 10.5). IR spectrum (chloroform): 3 611, 3 436, 1 052 (O–H); 1 719, 1 156, 1 306 (lactone). For $C_{27}H_{46}O_4$ (434.7) calculated: 74.61% C, 10.67% H; found: 74.15% C, 10.35% H.

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REFERENCES

1. Grove M. D., Spencer G. F., Rohwedder W. K., Mandava N., Worley J. F., Warthen J. D., jr., Steffens G. L., Flippen-Anderson J. L., Cook J. C., jr.: Nature *281*, 216 (1979).
2. Kohout L.: Collect. Czech. Chem. Commun. *59*, 457 (1994).
3. Wintersteiner O., Moore M.: J. Am. Chem. Soc. *66*, 1507 (1943).
4. Davies A. R., Summers G. H. R.: J. Chem. Soc., C *1967*, 1227.
5. Hodge P., Khan M. N.: J. Chem. Soc., Perkin Trans. 1 *1975*, 809.
6. Bridgeman J. E., Cherry P. C., Clegg A. S., Evans J. M., Jones E. R. H., Kasal A., Kumar V., Meakins G. D., Morisawa Y., Richards E. E., Woodgate P. D.: J. Chem. Soc., C *1970*, 250.
7. Mitchell J. W., Livingstone G. A.: *Methods of Studying Plant Hormones and Growth Regulating Substances*, p. 26, Agriculture Handbook No. 336. U.S. Government Printing Office, Washington, D.C. 1968.

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