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TRICHOTHECENES, 1: THE SYNTHESIS OF 4-DEOXYVERRUCAROL FROM VERRUCAROL AND DIACETOXYSCIRPENOL

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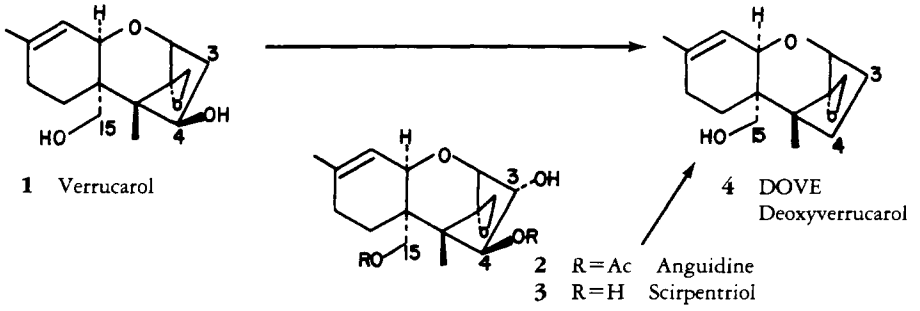
ABSTRACT.—4-Deoxyverrucarol (**4**) has been synthesized for use in studies for the preparation and development of monoclonal antibodies for trichothecenes. Both verrucarol (**1**) and anguidine (**2**) have been converted to deoxyverrucarol (DOVE) (**4**) by deoxygenation at C3 and at C3 and C4, respectively.

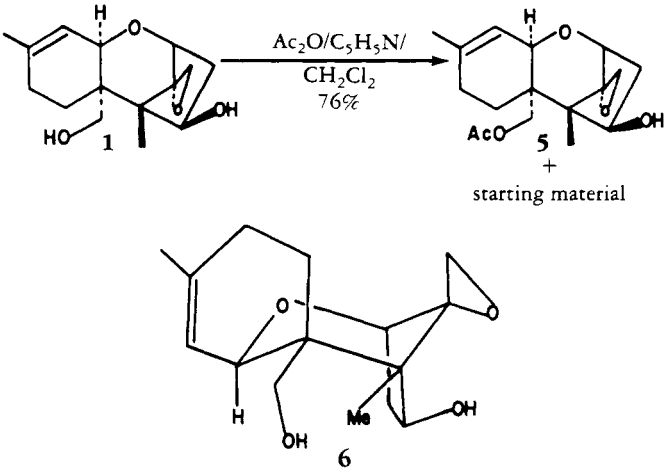
The extremely high acute toxicity of the trichothecenes has caused investigators to seek methods that would effectively dilute or nullify the toxic effects of these substances. Excellent reviews on the chemistry, biochemistry, and biology of tricothecenes have been published (1-6). The use of some of the known, naturally occurring trichothecenes in the trial development of monoclonal antibody technology for these systems has met with failure, presumably because of this high toxicity. As part of a continuing effort in our laboratories, we are attempting to prepare somewhat less toxic analogs of known trichothecenes that might prove to be more successful in this regard.

We reasoned that because verrucarol (**1**) was considerably less toxic than 4,15-diacetoxyscirpenol (anguidine) (**2**) and scirpenetriol (**3**), the next logical step to reducing the toxicity even further might be the removal of the C-4 oxygen functionality (7) of verrucarol (**1**) to give deoxyverrucarol (DOVE) (**4**) (8). Recently we have successfully been able to synthesize DOVE from both verrucarol and anguidine (Scheme 1). Deoxyverrucarol (**4**) was first prepared from verrucarol (**1**). Verrucarol was obtained by the basic hydrolysis of crude mixtures of roridins and verrucarins produced by *Myrothecium verrucaria*, followed by chromatography (9).



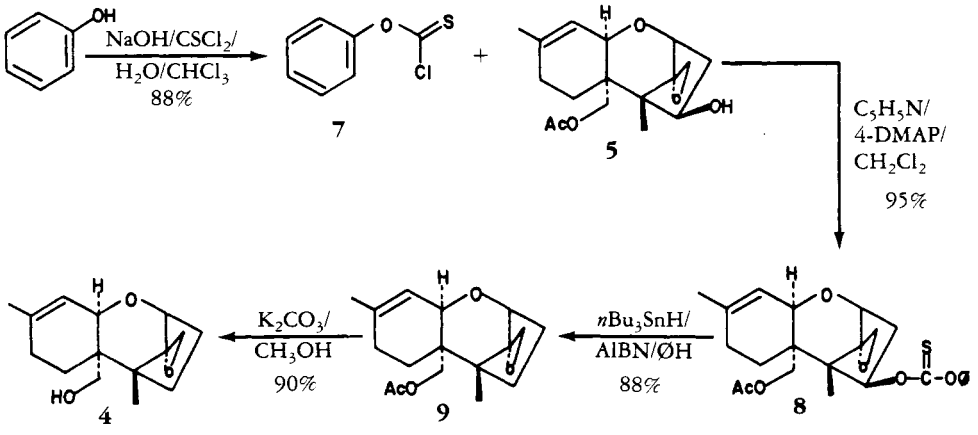
SCHEME 1

The first stage required differentiation of the C-4 and C-15 alcohols of compound **1**. The selective acylation of the C-15 primary alcohol has been accomplished by Fraser-Reid (10). In this manner, treatment of verrucarol (see Scheme 2) with Ac_2O-pyridine gave a 76% yield of the 15-acetoxyverrucarol (**5**) and some recovered verrucarol (**1**). This selectivity difference may be exacerbated because of effective shielding of the C-4 β-alcohol by the oxygen of the C-12, 13 epoxy group (**6**). In addition, the C-3 hydroxyl group of anguidine (**2**) has been removed by Frasier-Reid using this method (10).



SCHEME 2

The next stage involved the deoxygenation at C-4. The stannane reduction of thioesters (11) has been shown to be an exceptionally mild method for reducing alcohols to hydrocarbons. The moiety used for reduction in our study is the thionocarbonate. Phenylchlorothionocarbonate (**7**) is readily prepared (88%) by the reaction of sodium phenoxide with thiophosgene in aqueous $CHCl_3$ (12) (two-phase) (Scheme 3). Treatment of alcohol **5** with phenylchlorothionocarbonate and pyridine-4-dimethylaminopyridine gave an excellent (95%) yield of the thionocarbonate **8**. Subsequent reduction of **8** with tri-*n*-butyltinhydride (11) in C_6H_6 (AIBN) afforded 15-acetoxy-4-deoxyverrucarol (**9**) (88%). Hydrolysis of the C-15 acetate with K_2CO_3 in MeOH gave the target 4-deoxyverrucarol (DOVE) (**4**) in 90% yield.

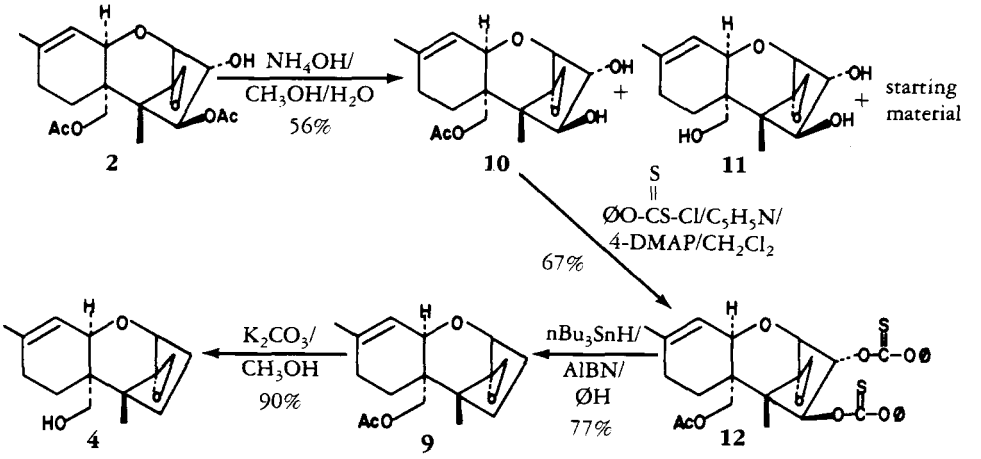


SCHEME 3

The lack of large readily available amounts of verrucarol (**1**) prompted us to develop an alternate route to DOVE (**4**) from anguidine (**2**). Anguidine (**2**) is easily obtained in crystalline form at concentrations of up to 290 mg/liter (13,14) from fermentation broths of *Fusarium* strains.

The conversion of **2** to **4** requires the selective protection of the C-15 oxygen, and the reductive removal of the C-3 α- and the C-4 β-oxygens either separately or concurrently. In practice, we found it easier to deprotect the C-4 acetate of anguidine (**2**) selectively, and then remove both the C-3 and C-4 alcohols simultaneously.

Treatment of anguidine (**2**) with aqueous NH_3 in MeOH (13), with careful monitoring of the reaction (tlc analysis), led to a mixture (Scheme 4) of diol (**10**), triol (**11**), and starting material (**2**), from which the desired diol (**10**) could be easily separated by chromatography in 56% yield. Tandem removal of the C-3 and C-4 hydroxy groups was accomplished by the same deoxygenation procedure used in the previous route. Diol **10** was treated with excess phenylchlorothionocarbonate (**7**)-pyridine-4-dimethylaminopyridine to give a 67% yield of the bisthionocarbonate **12**. Again, reduction of these groups to the hydrocarbons was effected by treatment of **12** with tri-*n*-butyltinhydride in C_6H_6, to afford 15-acetoxy-4-deoxyverrucarol (**9**). The material (**9**) prepared by this pathway was identical to the acetate prepared by the verrucarol pathway (Schemes 2 and 3). Additionally, this material was also readily hydrolyzed to DOVE (**4**) (90%) upon treatment with K_2CO_3 in MeOH.



SCHEME 4

In conclusion, we have shown that the thionocarbonates of alcohols at the 3 and 4 positions of the trichothecene system are readily reduced by tri-*n*-butyltinhydride to the corresponding hydrocarbon. In this way, we have prepared 4-deoxyverrucarol (DOVE) (**4**) by two routes: from verrucarol (**1**) and from anguidine (**2**). The DOVE (**4**) has undergone toxicity evaluation and conjugation for the possible production of monoclonal antibodies. The results of the toxicity study is shown in Table 1.

TABLE 1. Toxicity Studies of Selected Tricothecenes

Compound	LD_{50} Mouse (mg/kg)	IP_{50}[a] (μg/ml)	Skin MED[b] (ng)
DOVE (**4**)	≥164	62.2	≥500
Verrucarol	≥42.2	15.8	≥500
T-2 Toxin	3.29	0.005	50.2

[a]IP_{50}=50% inhibition of protein synthesis.
[b]MED=minimum effect dose.

EXPERIMENTAL

Melting points were obtained on a Fisher-Johns apparatus and are uncorrected. Pmr spectra were determined on a Varian Associates XL-100 or EM-360 spectrometer using TMS as an internal standard. Ir spectra were determined on a Perkin-Elmer 281 spectrometer as $CHCl_3$ solutions in 0.1 mm NaCl cells or as neat films on NaCl plates, using the polystyrene 1601 cm^{-1} band as calibration. Gas chromatographs were run on a Hewlett-Packard Model 5750 Research Chromatograph using the flame ionization mode. Tlc was performed on E. Merck glass supported silica gel 60 (0.25 mm, F-254) plates. Column chromatog-

raphy was done by the "flash chromatography" method of Still (E. Merck silica gel 60, 230-400 mesh) (15). Chromatographic purifications using Michel-Miller columns were of the mplc (medium pressure) type using Whatman LPS-1 (13-24 mm) silica gel and an FMI Model RP-SY Lab Pump operated between 50 and 150 psi.

PHENYLCHLOROTHIONOCARBONATE (**7**).—A solution of 5.00 g (0.0430 moles) of thiophosgene in 25 ml of $CHCl_3$ was cooled to 0° in an ice bath. A cold (0°) solution of 4.09 g of phenol (0.0430 moles) in 40 ml of 5% NaOH was added dropwise to the vigorously stirred, cold thiophosgene solution over a period of 10 min. After the addition was completed, the mixture was vigorously stirred at 0° for 2¼ h. Glc analysis indicated the complete disappearance of phenol and thiophosgene. The mixture was transferred to a separatory funnel and the $CHCl_3$ separated. The aqueous layer was extracted with 3×20 ml of $CHCl_3$. The combined organic layers were washed with 20 ml of 10% HCl, 20 ml of H_2O, dried ($MgSO_4$) and concentrated *in vacuo* to a clear, orange oil. The oil was distilled *in vacuo* using a short-path distillation apparatus to afford 6.58 g (88%) of phenylchlorothionocarbonate (**7**) as a clear, yellow oil, boiling point 87-92° (11 mm) (lit. 81-83°) [6 mm]). This material solidifies upon storing in the refrigerator and remelts when allowed to stand at room temperature. Pmr ($CDCl_3$; 60 MHz) δ 6.90-7.60 (m) ppm; ir (neat) 3060, 1479, 1240 cm^{-1}; tlc Rf=0.78 (3:2, EtOAc-hexanes); glc (6 ft., 10% UC-W98; recorder=0.25 in/min) retention time=9.25 min.

15-ACETOXYVERRUCAROL (**5**).—A solution of 0.500 g (0.0019 moles) of verrucarol (**1**) in 23 ml of dry CH_2Cl_2 and 0.86 ml (0.840 g; 0.0106 moles) of anhydrous pyridine was treated with 0.227 g (0.21 ml; 0.0022 moles) of Ac_2O. The mixture was stirred at room temperature under N_2 for 40 h. At this time, an additional 0.034 g (0.03 ml; 0.0003 moles) of Ac_2O was added and stirring continued for 24 h. The reaction mixture was diluted with 50 ml of CH_2Cl_2 and washed successively with 1×20 ml 10% HCl, 1×20 ml saturated $NaHCO_3$ solution and dried ($MgSO_4$). The volatiles were removed *in vacuo* and the residue chromatographed on 25 g of silica gel using 50% EtOAc in petroleum ether. This gave 80 mg of recovered verrucarol (**1**) and 0.440 g (76%) of 15-acetoxyverrucarol (**5**), mp 144-145° (lit. not reported). Pmr ($CDCl_3$); 60 MHz δ 0.88 (s, 3H), 1.40-2.50 (m, containing two 3H singlets at 1.67 and 2.06, 13H), 3.50-4.60 (m, 5H), 5.29 (d, J=4.4 Hz, 1H) ppm; ir ($CHCl_3$) 3500, 2980, 1735 cm^{-1}; tlc, Rf=0.45 (EtOAc).

15-ACETOXYVERRUCAROL-4-PHENYLTHIONOCARBONATE (**8**).—A solution of 0.440 g (0.0014 moles) of acetate (**5**) in 55 ml of dry CH_2Cl_2 containing 0.520 g (0.53 ml, 0.0066 moles) of pyridine and 0.185 g (0.0015 moles) of 4-dimethylaminopyridine was treated with 0.568 g (0.46 ml, 0.0033 moles) of phenylchlorothionocarbonate (**7**). The mixture was stirred at room temperature under N_2 for 20 h. Tlc analysis (3:2, hexanes-EtOAc) showed *ca.* 20% of starting material remaining. An additional 0.284 g (0.23 ml, 0.0017 moles) of thionocarbonate (**7**) was added at this time and stirring continued for 30 h more. The mixture was diluted to 100 ml with CH_2Cl_2 and washed with 2×30 ml 10% HCl, 1×20 ml saturated NaCl solution, dried ($MgSO_4$), and the volatiles evaporated *in vacuo*. The resulting oil was chromatographed on 25 g of silica gel using 25% EtOAc in petroleum ether as the eluant. This afforded 0.605 g (95%) of thionocarbonate (**8**) as an oil that slowly crystallized, mp 49-50°; pmr ($CDCl_3$; 60 MHz) δ 0.93 (s, 3H), 1.40-2.70 (m, containing two 3H singlets at 1.69 and 2.03, 12H), 2.77 (d, J=4.0 Hz, 1H), 3.07 (d, J=4.0 Hz, 1H), 3.50-3.87 (m, 2H), 3.95 (d, J=12.0 Hz, 1H), 4.12 (d, J=12.0 Hz, 1H), 5.30 (d, J=5.6 Hz, 1H), 6.07 (d of d, J_1=7.6 Hz, J_2=3.4 Hz, 1H), 6.78-7.42 (m, 5H) ppm; ir ($CHCl_3$) 3015, 1740 cm^{-1}; tlc, Rf=0.25 (3:2, hexanes-EtOAc); cims (NH_3)=445 (m+1).

15-ACETOXY-4-DEOXYVERRUCAROL (**9**).—*Method A. From 15-acetoxyverrucarol-4-phenylthionocarbonate* (**8**): A solution of 0.540 g (0.0012 moles) of thionocarbonate (**8**) and 0.052 g of 2,2′-azobis(2-methylpropionitrile) (AIBN) in 156 ml of dry C_6H_6 was heated at 75° under N_2. At this time, 2.100 g (1.94 ml, 0.0072 moles) of *n*-Bu_3SnH was added all at once via syringe. Heating at 75° under N_2 was continued for 20 h. Tlc analysis (3:2, hexanes-EtOAc) showed that *ca.* 20% of the starting material remained. An additional 0.025 g, of AIBN and 1.08 g (1.00 ml, 0.0037 moles) of *n*-Bu_3SnH was added and stirring at 75° was continued for another 26 h. The reaction mixture was cooled and the C_6H_6 removed *in vacuo*. Aqueous (5%) CH_3CN (100 ml) was added to the residue and the resulting solution was washed with 5×20 ml of pentane to remove most of the alkyl tin compounds. The CH_3CN layer was dried ($MgSO_4$) and the volatiles evaporated *in vacuo*. The resulting oil was chromatographed on 25 g of silica gel using petroleum ether (500 ml) followed by 15% EtOAc in petroleum ether (500 ml) as the eluants, to afford 0.308 g (88%) of 15-acetoxy-4-deoxyverrucarol (**9**) as a very viscous, colorless oil. Pmr ($CDCl_3$; 100 MHz) δ 0.83 (s, 3H), 1.50-2.20 (m, containing two 3H singlets at 1.72 and 2.05, 14H), 2.88 (d, J=4.0 Hz, 1H), 3.17 (d, J=4 Hz, 1H), 3.60-3.78 (m, 2H), 3.88 (d, J=12.0 Hz, 1H), 4.12 (d, J=12.0 Hz, 1H), 5.44 (d, J=5.0 Hz, 1H) ppm; ir ($CHCl_3$) 2950, 1738 cm^{-1}; tlc, Rf=0.44 (3:2, hexanes-EtOAc); high resolution ms obsvd, *m/z* 292.1692, $C_{17}H_{24}O_4$ requires 292.1674.

Method B. From 15-acetoxyscirpen-3,4-(bis-phenylthionocarbonate) (**12**): A solution of 0.340 g (0.0006

moles) of bis-phenylthionocarbonate (**12**) and 0.060 g of AIBN in 75 ml of dry C_6H_6 was heated at 80°. At this time, 1.66 g (1.53 ml, 0.0057 moles) of *n*-Bu_3SnH was added all at once, and heating at 80° under N_2 was continued for 48 h. Tlc analysis (3:2, hexanes-EtOAc) showed no remaining starting material. The reaction mixture was cooled to room temperature, and the C_6H_6 was removed *in vacuo*. Aqueous (5%) CH_3CN (50 ml) was added to the residue, and this solution was washed with 5×20 ml of pentane to remove the alkyl tin compounds. The CH_3CN layer was dried ($MgSO_4$) and the volatiles evaporated *in vacuo*. The residual oil was chromatographed on a 25 g Michel-Miller silica gel column using petroleum ether (500 ml) followed by 15% EtOAc in petroleum ether (500 ml) as the eluants, to afford 0.127 g (77%) of 15-acetoxy-4-deoxyverrucarol (**5**). The physical and spectroscopic properties of (**5**) prepared in this way (Method B) were identical to those of the sample obtained by the procedures of Method A.

15-ACETOXYSCIRPEN-3,4-DIOL (**10**).—A solution of 1.500 g (0.0041 moles) of diacetoxyscirpenol (**2**) (anguidine) in 150 ml of MeOH was treated with 150 ml of 1 M NH_4OH. The reaction mixture was stirred at room temperature and carefully monitored by tlc analysis using 20% Me_2CO in CH_2Cl_2 as the eluant. After 24 h, analysis showed a mixture of starting material (**2**), 15-acetoxyscirpen-3,4-diol (**10**), and 3,4,15-scirpentriol (**11**). The volatiles were removed completely *in vacuo* and the resulting oil purified by chromatography on a 150 g Michel-Miller silica gel column using 2% MeOH in $CHCl_3$ as the eluant. This afforded a small amount (0.100 g) of starting material, 0.748 g (56%) of 15-acetoxyscirpen-3,4-diol (**10**) mp 170-171° (lit. 170-172°), and 0.40 g (35%) of scirpentriol (**11**) mp 188-189° (lit. 189-191°). Pmr ($CDCl_3$, 100 MHz) δ 0.78 (s, 3H), 1.60-2.20 (m, containing two 3H singlets at 1.70 and 2.04, 10H), 2.74 (d, J=4.2 Hz, 1H), 3.02 (d, J=4.2 Hz, 1H), 3.62 (d, J=4.8 Hz, 1H), 3.70-4.60 (m, 7H), 5.51 (d, J=4.8 Hz, 1H) ppm; ir ($CHCl_3$) 3440, 2970, 1734 cm^{-1}; tlc, Rf=0.13 (20% Me_2CO in CH_2Cl_2).

15-ACETOXYSCIRPEN-3,4-(BIS-PHENYLTHIONOCARBONATE) (**12**).—A solution of 0.275 g (0.0009 moles) of 15-acetoxyscirpen-3,4-diol (**10**) in 15 ml of dry CH_2Cl_2 containing 0.427 g (0.44 ml, 0.0054 moles) of pyridine and 0.132 g (0.0011 moles) of 4-dimethylaminopyridine was treated all at once with 0.429 g (0.44 ml, 0.0054 moles) of phenylchlorothionocarbonate (**7**). The reaction mixture was stirred under N_2 at room temperature for 20 h. Tlc analysis (20% Me_2CO in CH_2Cl_2) showed that no starting material remained. The reaction mixture was diluted to 75 ml with CH_2Cl_2 and washed with 2×25 ml 10% HCl, 1×25 ml saturated NaCl solution, dried ($MgSO_4$) and the volatiles evaporated *in vacuo*. The yellow residue was chromatographed on a 25 g Michel-Miller silica gel column using 25% EtOAc in petroleum ether as the eluant. This afforded 0.341 g (68%) of bis-thionocarbonate (**12**) as a glassy foam. Pmr ($CDCl_3$; 60 MHz) δ 0.95 (s, 3H), 1.50-2.05 (m, containing two 3H singlets at 1.73 and 2.04, 10H), 2.77 (d, J=4.0 Hz, 1H), 3.05 (d, J=4.0 Hz, 1H), 3.75-4.50 (m, 4H), 5.41 (d, J=4.4 Hz, 1H), 5.84 (m, 1H), 6.36 (m, 1H), 7.20-7.50 m, 5H) ppm; ir ($CHCl_3$) 2130, 1740 cm^{-1}; tlc, Rf=0.73 (EtOAc); cims (NH_3)=597 (M+1).

4-DEOXYVERRUCAROL (DOVE) (**4**).—A stirred solution of 0.275 g (0.0009 moles) 15-acetoxy-4-deoxyverrucarol (**9**) in 30 ml of anhydrous MeOH was treated with 0.700 g (0.0051 moles) of anhydrous K_2CO_3. The reaction mixture was stirred at room temperature for 4.5 h. The volatiles were evaporated *in vacuo*, and 100 ml of CH_2Cl_2 was added to the residue. The organic layer was washed with 1×30 ml H_2O, 1×30 ml saturated NaCl solution, dried ($MgSO_4$) and evaporated *in vacuo* to leave an off-white solid. The solid was triturated with 2 ml of petroleum ether (0°) to give 0.217 g (92%) of 4-deoxyverrucarol (**4**), mp 122-123°. Pmr ($CDCl_3$; 100 MHz) δ 0.93 (s, 3H), 1.07-2.33 (m, containing 3H singlet at 1.72, 12H), 2.89 (d, J=4.6 Hz, 1H), 3.17 (d, J=4.6 Hz, 1H), 3.50 (d, J=12 Hz, 1H), 3.56-3.80 (m, 3H), 5.44 (d, J=4.6 Hz, 1H) ppm; ir ($CHCl_3$) 3470, 2980 cm^{-1}; ms *m/z* 250 (M+), 219 (-CH_2OH); tlc Rf=0.20 (3:2, hexanes-EtOAc); high resolution ms, obsd. *m/z* 250.1542, $C_{15}H_{22}O_3$ requires 250.1569.

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