(37) Spedding, F. H., Beaudry, B. J., Henderson, D. C., Moorman, J., *J. Chem. Phys.*, **60**, 1578 (1974).
(38) Spedding, F. H., Henderson, D. C., *J. Chem. Phys.*, **54**, 2476 (1971).
(39) Sommers, J. A., Westrum, E. F., *Proc., Rare Earth Res. Conf. 12th*, 433 (1976).
(40) Stull, D. R., Prophet, H., *Natl. Stand. Ref. Data Ser., Natl. Bur. Stand.*, **No. 37** (1971).
(41) Suvorov, A. V., Krzhizhanovskaya, Novikov, G. I., *Russ. J. Inorg. Chem.*, **11**, 1441 (1966).
(42) Walden, G. E., Smith, D. F., *U.S., Bur. Mines, Rep. Invest.*, **No. 5859** (1961).
(43) Westrum, E. E., *Adv. Chem. Ser.*, **No. 71**, 25 (1967).
(44) Westrum, E. F., Beale, A. F., Jr., *J. Phys. Chem.*, **65**, 353 (1961).
(45) Zmbov, K. F., Margrave, J. L., *Adv. Chem. Ser.*, **No. 72**, 267 (1968).

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NEW COMPOUND SECTION

Synthesis of 5β-Chol-7-en-6-one Analogues of Ecdysone from Cholic Acid

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Starting with cholic acid, syntheses of B-ring 5β-enone steroid systems are delineated. Both methyl 3α,12α-diacetoxy-6-oxo-5β-chol-7-en-24-oate and methyl 3α,12α-dinitroxy-6-oxo-5β-chol-7-en-24-oate were inactive as insect moulting hormones. An interesting comparison between the selective synthesis and nonselective synthesis of methyl 3α,12α-diacetoxy-6-oxo-5β-chol-7-en-24-oate is made.

During the course of our pilot studies directed toward synthesis of quassin derivatives, we had a need for steroids containing the B-ring 5β-enone system characteristic of ecdysone, an insect moulting hormone. (For examples of the author's prior work with other enone systems ref 4.) Since these B-ring 5β-enone systems may provide a potentially useful starting point for elaborating other ecdysone analogues (*7*), this paper summarizes their synthesis.

Results and Discussion

The starting material, 3α,12α-dinitroxy-7α-hydroxy-5β-cholan-24-oic acid (**1**), was synthesized by a modified procedure (Scheme I) (*5, 10*). The methyl ester **2** was dehydrated with $POCl_3$ in pyridine under anhydrous conditions to yield exclusively **3**; if the methyl ester **2** is not scrupulously dried some 7β-chloro substituted product is also obtained. Allylic oxidation (*2*) of olefin **3** with excess CrO_3·2py provided enone **4**. Selective removal of the nitroxy groups by Zn dust reduction in glacial acetic acid below room temperature (*6*) followed by acetylation in pyridine yielded ene **5**. This olefin was subsequently oxidized as above to give enone **6**.

Preliminary attempts to selectively reduce the 6-oxo group in 5β-enone **6** with $NaBH_4$ in CH_3OH–THF containing ethyl acetate led to only recovered starting material. By contrast the 6-oxo group in the 5α-enone system is easily reduced under these conditions (*8*). Interestingly, if one compares the nitroxy vs. acetoxy analogues, one observes that the melting points, R_f values, and C-19 and C-18 methyl ^1H NMR chemical shifts are higher for the nitroxy analogues (compare **3** vs. **5** and **4** vs. **6**). Enones **4** and **6** were inactive as insect moulting hormones when fed admixed with food to lepidopterous larvae (cf. with acknowledgment).

In the conversion of the dinitroxy olefin **3** to diacetoxy olefin **5** in one run yielded a side product which was subsequently identified as methyl 3α,12α-diacetoxy-7β-chloro-5β-cholan-24-oate. Initially this side product was thought to be methyl 3α,12α-diacetoxy-5β-chol-8(14)-en-24-oate (**10**) because of the conspicuous absence of olefinic protons in its ^1H NMR spectrum. Thus, an attempt to synthesize olefin **10** (Scheme II) by refluxing cholic acid (50 g) with fused $ZnCl_2$ (50 g) in acetone for 0.25 h (*3*) followed by acetylation and esterification with diazomethane yielded instead a small quantity (10 g) of crude olefin **5** isolated by column chromatography through silica gel and a substantial amount of methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate (50 g). Oxidation of this crude olefin **5** (10 g) as per Scheme I, yielded 4.5 g of enone **6**. Prolonged (2.5 h) refluxing of cholic acid with fused $ZnCl_2$ in acetone followed by acetylation and esterification did result in the corresponding ester **10** of the reported apocholic acid **8** (*3*). These experiments emphasize the importance of the duration of refluxing (not reported in ref 3) in order to completely dehydrate and subsequently isomerize cholic acid to apocholic acid. In addition, if one compares the overall yields of enone **6** in the selective synthetic route (Scheme I) vs. the nonselective route (Scheme II), one obtains 25 and 9%, respectively. However, since the methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate can be hydrolyzed back to cholic acid for recycling through Scheme II, this latter synthetic route becomes economically preferred to Scheme I.

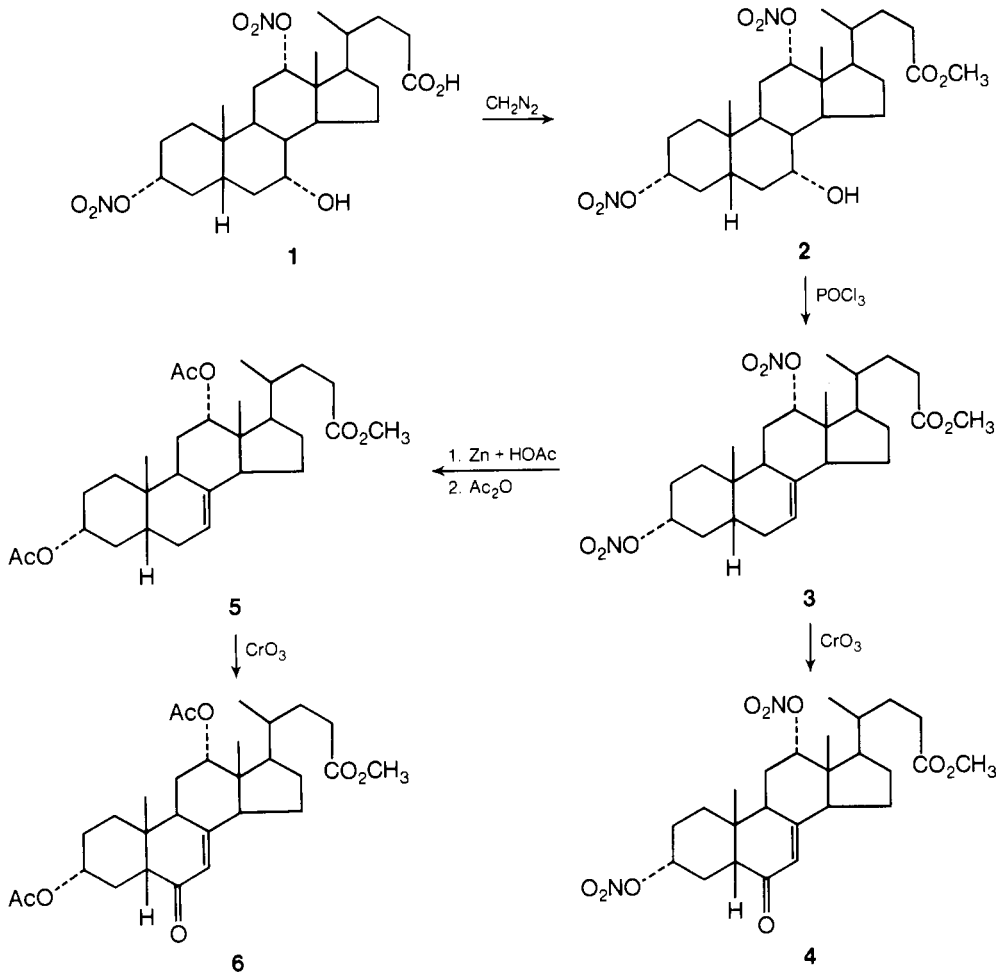
Experimental Section

General Procedure. All melting points were determined with a Fisher-Johns apparatus and are corrected. IR data reported in inverse centimeters (cm^{-1}) were obtained in $CHCl_3$ solution against a blank; ^1H NMR data, reported in ppm (δ) from Me_4Si, were obtained in $CDCl_3$ on a Varian A-60 or T-60 instrument; and mass spectra were obtained at an ionization voltage of 70 eV with a Nuclide 12-90-G single-focusing instrument having a resolution capability of 10 000.

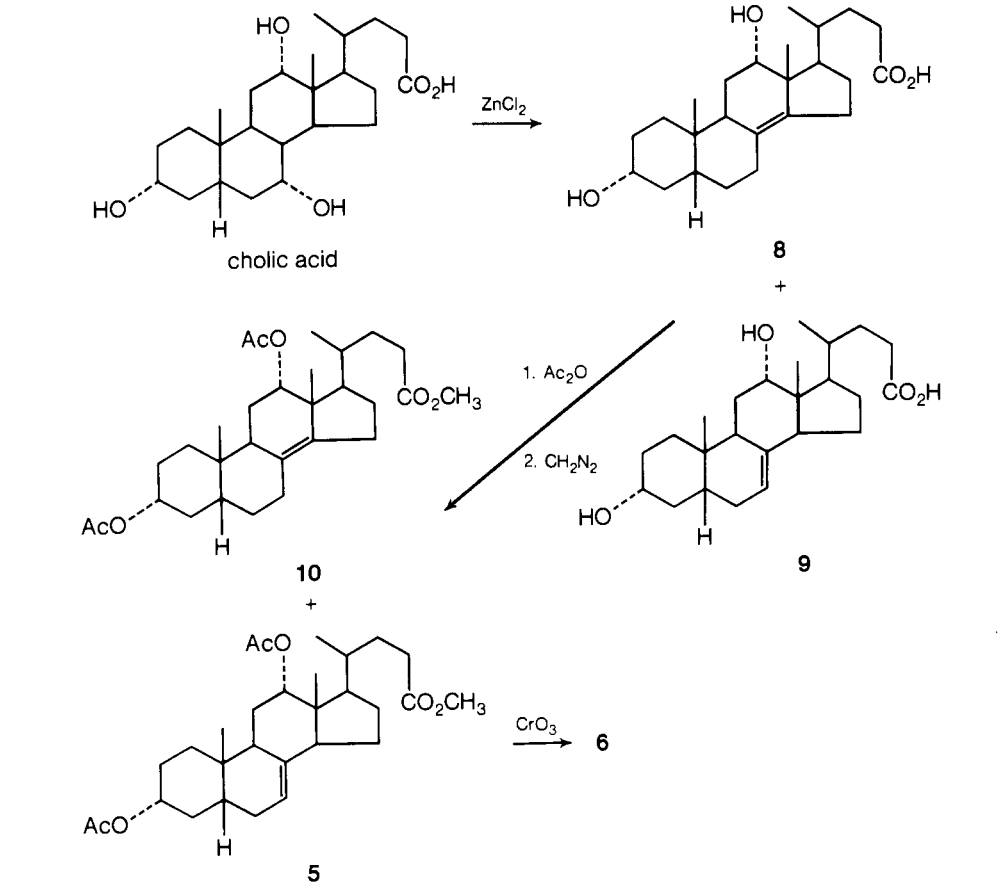
Column chromatography was performed using silica gel (MCB Grade 62) and TLC was performed on silica gel HF_{254} (E. Merck); the latter were usually developed with 1:1 or 4:1 hexane–EtOAc. Visualization of the TLC was effected by spraying with 2% ceric sulfate in 2 N sulfuric acid followed by brief heating; **4** and **6** gave the characteristic yellow color of enones (*4*). All reactions were monitored by TLC.

***3α,12α-Dinitroxy-7α-hydroxy-5β-cholan-24-oic* (1).** A mixture of cholic acid (100 g), benzene (660 mL), pyridine (170 mL), and acetic anhydride (170 mL) was allowed to stand at room temperature for 20 h (*5*). The mixture was poured into water and the benzene layer was washed several times with dilute HCl. The

Scheme I



Scheme II



solvent was removed on a rotary evaporator to yield 120 g of 3α,7α-diacetoxy-12α-hydroxy-5β-cholan-24-oic acid of which a small portion was recrystallized from CH_3OH–$CHCl_3$: mp 262–263 °C; $\bar{\nu}_{max}$ 3450 (broad acid OH str), 1738 and 1250 (OAc), and 1710 cm^{-1} (acid C=O str); ^1H NMR δ 4.87 (peak, 1p, 7β-H), 4.52 (hump, 1p, 3β-H), 4.00 (peak, 1p, 12β-H), 2.20 (m, 2p, C-23), 2.03 and 2.00 (2s, 3p each, 3αOAc and 7α-OAc), 0.93 (s, 3p, C-19), and 0.70 (s, 3p, C-18). This diacetoxy cholic acid (120 g) was dissolved in absolute CH_3OH (1.4 L) and acetyl chloride (26 mL) was added. After 36 h at room temperature, ice cold H_2O was added until the solution was turbid; when much of the ester had crystallized, the supernatant was further diluted with H_2O (3 L total volume) and allowed to stand for 1 h more until crystallization was complete. This yielded 108 g of methyl 3α,12α-dihydroxy-7α-acetoxy-5β-cholan-24-oate: $\bar{\nu}_{max}$ 3400 (OH str), 1740, 1730, and 1250 cm^{-1} (OAc and CO_2CH_3); ^1H NMR δ 4.87 (peak, 1p, 7β-H), 3.98 (peak, 1p, 12β-H), 3.65 (s, 3p, OCH_3), 3.40 (hump, 1p, 3β-H), 2.25 (m, 2p, C-23), 2.07 (s, 3P, 7α-OAc), 0.92 (s, 3p, C-19), and 0.69 (s, 3p, C-18). This diol (108 g) was dissolved in $CHCl_3$ (600 mL) and fuming nitric acid reagent (prepared by combining 450 mL of fuming HNO_3, specific gravity 1.6, with 1070 mL of Ac_2O at −5 °C) (*10*) was slowly added while maintaining the temperature at −5 °C. After stirring for 1 h, the reaction mixture was poured into ice water and the separated chloroform layer was washed with $NaHCO_3$ solution. Removal of the solvent on a rotary evaporator afforded methyl 3α,12α-dinitroxy-7α-acetoxy-5β-cholan-24-oate (100 g) which was eluted through silica gel (1200 g deactivated with 15% H_2O) with $CHCl_3$ containing 5% CH_3OH: $\bar{\nu}_{max}$ 1740, 1720, and 1240 (OAc and CO_2CH_3) and 1630, 1280, 860, and 760 cm^{-1} (ONO_2); ^1H NMR δ 5.30 (peak, 1p, 7β-H), 4.92 (peak, 1p, 12β-H), 4.80 (hump, 1p, 3β-H), 3.67 (s, 3p, OCH_3), 2.20 (m, 2p, C-23), 2.08 (s, 3p, 7α-OAc), 0.89 (s, 3p, C-19), and 0.82 (s, 3p, C-18). This purified material was dissolved in CH_3OH (2.5 L) containing KOH (140 g). After refluxing for 5 h, the reaction mixture was poured into ice (4 kg) containing concentrated HCl (1.2 L). The precipitate was collected by filtration to yield 98 g of 3α,12α-dinitroxy-7α-hydroxy-5β-cholan-24-oic acid as a yellow solid. Recrystallization of a portion yielded colorless prismatic needles: mp 200–201 °C (lit. (*10*) mp 198–199 °C); $\bar{\nu}_{max}$ 3425 (broad acid OH str), 1715 (acid C=O str), and 1630, 1280, 870, and 760 cm^{-1} (ONO_2); ^1H NMR δ 6.15 (peak, 2p, OH which vanishes with D_2O shake), 5.28 (peak, 1p, 12β-H), 4.70 (hump, 1p, 3β-H), 3.90 (peak, 1p, 7β-H), 0.95 (s, 3p, C-19), and 0.83 (s, 3p, C-18).

Methyl 3α,12α-Dinitroxy-7α-hydroxy-5β-cholan-24-oate (2). An ether solution (2 L) of acid **1** (82 g of crude material) was treated with diazomethane. The ether was removed by evaporation and the residue was eluted through silica gel (800 g deactivated with 15% H_2O) with $CHCl_3$ containing 5% CH_3OH to yield 60 g of pure methyl ester **2**: 93–95 °C (fine colorless needles from ether–hexane); $\bar{\nu}_{max}$ 3400 (OH str), 1738 and 1250 (CO_2CH_3), and 1630, 1270, 860, and 760 cm^{-1} (ONO_2); ^1H NMR δ 5.27 (peak, 1p, 12β-H), 4.74 (hump, 1p, 3β-H), 3.87 (peak, 1p, 7β-H), 3.67 (s, 3p, OCH_3), 2.20 (m, 2p, C-23), 0.97 (s, 3p, C-19), and 0.83 (s, 3p, C-18).

Methyl 3α,12α-Dinitroxy-5β-chol-7-en-24-oate (3). A solution of ester **2** (36 g) in pyridine (500 mL) was treated with $POCl_3$ while chilling on an ice bath. After stirring at room temperature for 6 h, the amber mixture was poured into ice, and the organic product was taken up in benzene. The benzene layer was washed with water and concentrated. The residue was chromatographed through silica gel (800 g deactivated with 15% H_2O) with $CHCl_3$ containing 5% CH_2OH to yield 31 g of **3**: mp 133–134 °C (granular crystals from CH_3OH–H_2O); $\bar{\nu}_{max}$ 1738 (CO_2CH_3) and 1620, 1280, 865, and 760 cm^{-1} (ONO_2); ^{1}NMR δ 5.37 (peak, 1p, 7β-H), 5.22 (peak, 1p, 12β-H), 4.87 (hump, 1p, 3β-H), 3.63 (s, 3p, OCH_3), 2.25 (m, 4p, C-6 and C-23), 0.91 (s, 3p, C-19), and 0.70 (s, 3p, C-18).

Methyl 3α,12α-Dinitroxy-6-oxo-5β-chol-7-en-24-oate (4). Into stirring CH_2Cl_2 (300 mL) containing pyridine (88 mL) was added CrO_3 (54 g) (*9*). After stirring for 15 min, olefin **3** (11.0 g) dissolved in a minimum quantity of CH_2Cl_2 was added to the burgandy-colored reaction mixture. After stirring at room temperature for 18 h, another portion of CrO_3·2py slurry (made by adding 33 g CrO_3 to 54 mL of pyridine in 200 mL of CH_2Cl_2) was added. After another 7 h of stirring, the reaction mixture was decanted, the dark residue was washed with ether, and the combined organic layer was successively washed with 5% KOH, 5% HCl, 5% $NaHCO_3$, and NaCl solution. Removal of the Na_2SO_4 dried solvent yielded 9.8 g of residue which was chromatographed through silica gel to yield 7.3 g of enone **4**: mp 168–169 °C (needle clusters from hexane–EtOAc); $\bar{\nu}_{max}$ 1733 and 1240 (CO_2CH_3), 1665 (C=CC=O), 1630, 1280, and 860 cm^{-1} (NO_2); ^1H NMR δ 5.72 (peak, 1p, 7-H), 5.30 (peak, 1p, 12β-H), 4.90 (hump, 1p, 3β-H), 3.64 (s, 3p, OCH_3), 2.65 (m, 4p, C-9 and C-14 and C-23), 1.00 (s, 3p, C-19), and 0.78 (s, 3p, C-18); λ_{max} 243 (log ϵ_{max} 4.22).

Methyl 3α,12α-Diacetoxy-5β-chol-7-en-24-oate (5). Zn powder (140 g) was added in increments to a solution of ester (33 g) dissolved in glacial HOAc (1350 mL) maintained at 10–17 °C. After stirring for 45 min, the unreacted Zn and $Zn(OAc)_2$ precipitate was filtered off and washed with benzene. The HOAc filtrate was poured into H_2O and the precipitate was taken up with benzene which was washed well with dilute $NaHCO_3$ solution. Evaporation of the benzene solvent yielded 28 g of a glassy solid which was dissolved in pyridine (300 mL) and acetic anhydride (150 mL). After standing at room temperature for 24 h, the reaction mixture was poured into water which was subsequently extracted with benzene. The benzene solution was washed with H_2O and evaporated. Column chromatography through silica gel eluting with hexane–acetone (gradient) gave 23 g of ene **5**: mp 68–70 °C (lit. (*1*) mp 70–72 °C); ν_{max} 1738 and 1250 (OAc) and 1725 cm^{-1} (CO_2CH_3); ^1H NMR δ 5.13 (peak, 2p, C-6 and 12β-H), 4.87 (hump, 1p, 3β-H), 3.61 (s, 3p, OCH_3), 2.3 (m, 4p, C-5 and C-23), 2.10 and 2.00 (2s, 3p each, 3α-OAc and 12α-OAc), 0.85 (s, 3p, C-19), and 0.63 (s, 3p, C-18); *m/e* (%) 488 (2%, M^+), 428 (16%, M − HOAc), 368 (55%, M − 2HOAc), 348 (50%), 313 (58%, M − HOAc − $C_6H_{11}O_2$), 289 (91%), and 253 (100%, M − 2HOAc − $C_6H_{11}O_2$).

In one run from crude ester **3** synthesized from poorly dried **2**, a quantity of methyl 3α,12α-diacetoxy-7β-chloro-5β-cholan-24-oate was obtained: mp 163–165 °C (prisms from hexane–EtOAc); ν_{max} 1740 and 1250 (OAc) and 1725 cm^{-1} (CO_2CH_3); ^1H NMR δ 5.06 (peak, 1p, 12β-H), 4.64 (hump, 1p, 3β-H), 3.88 (hump, 1p, 7α-H), 3.67 (s, 3p, OCH_3), 2.09 and 2.02 (s, 3p each, 3α-OAc and 12α-OAc), 0.97 (s, 3p, C-19), and 0.78 (s, 3p, C-18); *m/e* (%) 466 and 464 (6 and 2, M − HOAc), 428 (10, M − HOAc − HCl), 406 and 404 (9 and 3, M − 2HOAc), 368 (12, M − 2HOAc − HCl), 351 and 349 (32 and 7, M − HOAc − $C_6H_{11}O_2$), 313 (14, M − HOAc − $C_6H_{11}O_2$ − HCl), 291 and 289 (27 and 9, M − 2HOAc − $C_6H_{11}O_2$), and 253 (29, M − 2HOAc − $C_6H_{11}O_2$ − HCl).

Methyl 3α,12α-Diacetoxy-6-oxo-5β-chol-7-en-24-oate (6). Dry CrO_3 (91 g) was slowly added to a magnetically stirred solution of pyridine (148 mL) and CH_2Cl_2 (370 mL) while chilling on a salt–ice bath. To this reaction mixture was added ene **5** (18.5 g) dissolved in CH_2Cl_2 (50 mL). After stirring at room temperature for 17 h, another portion of CrO_3·2py complex (prepared by adding 55 g of CrO_3 in 89 mL of pyridine and 300 mL of CH_2Cl_2) was added, and the reaction was stirred another 7 h. The dark reaction mixture was decanted into ether, and the tarry residue remaining in the flask was dissolved in $NaHCO_3$ solution and washed with ether several times. The ether layers were combined and successively washed with 5% KOH solution, 5% HCl solution, 5% $NaHCO_3$ solution, and NaCl solution. The Na_2SO_4 dried ether solution was concentrated and the resulting residue (15.2 g) was column chromatographed through silica gel (deactivated with 15% H_2O) by eluting with 5% CH_3OH in $CHCl_3$

to yield enone **6** (11.0 g): mp 128–130 °C (recrystallized from CH_3OH); ν_{max} 1740 and 1250 (OAc), 1720 (CO_2CH_3), and 1660 and 1630 cm^{-1} (C=CC=O); ^1H NMR δ 5.69 (peak, 1p, C-7), 5.26 (peak, 1p, 12β-H), 4.69 (hump, 1p, 3β-H), 3.66 (s, 3p, OCH_3), 2.8 (m, 4p, C-9, C-14, C-23), 2.17 and 2.00 (2S, 6p, 3α-OAc and 12α-OAc), 0.93 (S, 3p, C-19), and 0.71 (s, 3p, C-18); λ_{max} 243 (log ϵ_{max} 4.28); *m/e* (%) 502 (9, M^+), 442 (62, M − HOAc), 382 (100, M − 2HOAc), 327 (36, M − HOAc − $C_6H_{11}O_2$), and 267 (98, M − 2HOAc − $C_6H_{11}O_2$).

Anal. Calcd for $C_{29}H_{42}O_7$: C, 69.30; H, 8.42; O, 22.28. Found: C, 69.01; H, 8.42; O, 22.47.

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Literature Cited

(1) Berner, E., Lardon, A., Reichstein, T., *Helv. Chim. Acta*, **30**, 1549 (1947).
(2) Dauben, W. G., Lorber, M., Fullerton, D. S., *J. Org. Chem.*, **34**, 3587 (1969).
(3) Devor, A., Marlow, H., *J. Am. Chem. Soc.*, **68**, 2101 (1946).
(4) Dias, J. R., *J. Org. Chem.*, **39**, 1767 (1974); *Org. Mass Spectrom.*, **11**, 333 (1976).
(5) Feiser, L., Rajagopolan, S., *J. Am. Chem. Soc.*, **72**, 5530 (1950).
(6) Hodosan, F., Jude, I., Serban, N., *Chem. Ber.*, **95**, 1094 (1962).
(7) Kinnear, J., Martin, M., Horn, D., Middleton, E., Wilkie, J., Galbraith, M., Willing, R., *Aust. J. Chem.*, **29**, 1815 (1976).
(8) Nassim, B., M.S. Thesis, University of Missouri, Kansas City, 1975.
(9) Ratcliffe, R., Robehorst, R., *J. Org. Chem.*, **35**, 4000 (1970).
(10) Tanasecu, I., Hodosan, F., Jude, I., *Chem. Ber.*, **91**, 799 (1958).

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Preparation and Physical Properties of Some Desoxybenzoins and Isoflavones

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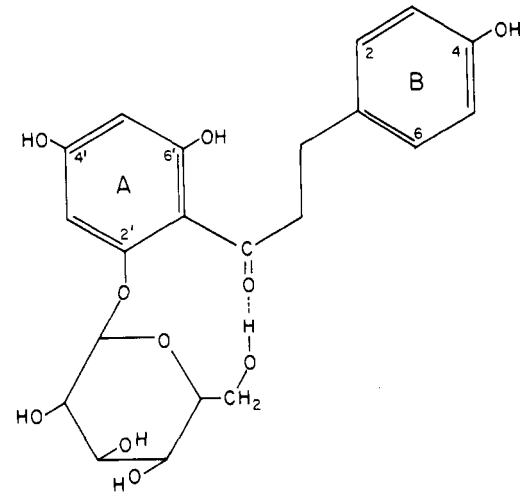
Simple esters of 2′,4′,6′-trihydroxyphenyl 4-nitrobenzyl ketone cannot be obtained when this desoxybenzoin is reacted with acetic anhydride or benzoyl chloride under mild conditions normally employed to acylate phenols. Instead, C_2-methyl or C_2-phenyl substituted isoflavones and their respective acetate and benzoate derivatives are formed. The IR and NMR spectral data as well as other physical properties of this series of flavonoids are reported.

The dihydrochalcone glucoside, phlorizin (I), is a valuable pharmacological tool for investigators studying membrane transport phenomena. It is a potent competitive inhibitor of the presumed receptor protein which facilitates the movement of glucose across cell membranes. A series of phlorizin analogues was prepared (*6*) in order to determine the structural features essential for I to interact with this membrane receptor in an in vitro intestinal (*7*) and an in situ renal (*13*) test system. The results of these studies suggested that the drug–receptor interaction depends, in part, on the formation of a strong hydrogen bond through the *p*-hydroxyl group on the B ring of phlorizin. In order to further investigate this interaction, some additional phlorizin derivatives were required especially one in which the critical nature and intramolecular spacing of this phenolic group could be tested. An appropriately substituted phenyl benzyl ketone (desoxybenzoin; II) was considered to be one of several aglycones that would be a suitable starting material to prepare 4-nitro- and 4-amino-substituted phlorizin-like test compounds. In order to form 2′-β-glycosides of II, the more acidic *p*-hydroxyl group had to be transiently protected and it was at this juncture, during routine attempts to acylate II in the 4′-position, that the



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exceptional reactivity of the methylene group in this compound was realized. When we used conditions comparable with those employed to partially acetylate or benzoylate the analogous phenyl methyl ketone, phloracetophenone (*5*), no simple 4′-ester of II could be isolated; instead acylation first occurs at the α-position to form III which spontaneously undergoes a Baker-Venkataraman type rearrangement (*1, 12*) to form the C_2-substituted isoflavone, IV. Although IV, as the free phenol, is the first