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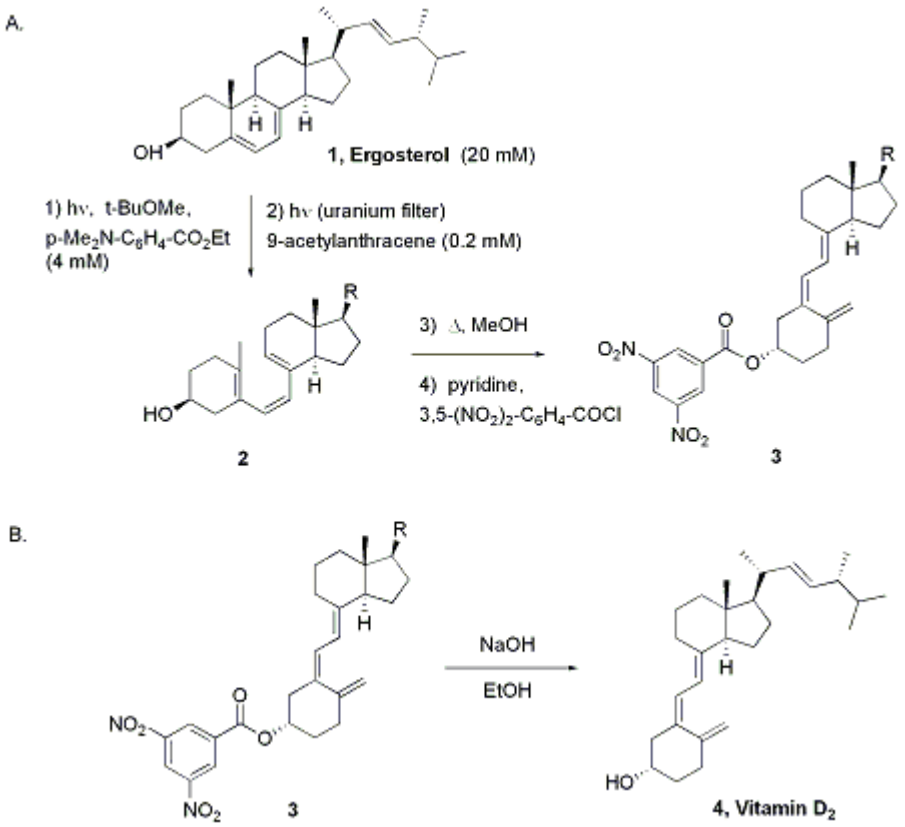
September 2014: The paragraphs above replace the section "Handling and Disposal of Hazardous Chemicals" in the originally published version of this article. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

DOI:10.15227/orgsyn.076.0275

Organic Syntheses, Coll. Vol. 10, p.718 (2004); Vol. 76, p.275 (1999).

VITAMIN D_2 FROM ERGOSTEROL

[9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol,(3β)- from Ergosta-5,7,22-trien-3-ol, (3β)-]



Submitted by Masami Okabe[1]
Checked by Gilles Chambournier and David J. Hart.

1. Procedure

Caution! Light from a mercury lamp is damaging to the eyes and skin. Suitable precautions, such as wearing an appropriate face shield, UV radiation protective eyewear and gloves, and surrounding the reaction vessel with aluminum foil, should be taken. It is recommended that one work in a hood and that the hood sash be covered with aluminum foil.

A. 3,5-Dinitrobenzoate of vitamin D_2 (**3**). A 2-L photo-reaction vessel equipped with a quartz immersion well, a thermometer, an argon-inlet tube, a mineral oil outlet-bubbler, a mechanical stirrer, and supported in an adequately sized Dewar (Note 1), is charged with 13.5 g (34 mmol) of ergosterol (**1**) (Note 2), 1.31 g (6.8 mmol) of ethyl p-dimethylaminobenzoate , and 1.7 L of tert-butyl methyl ether (tert-BuOMe) (Note 3). The mixture is stirred at room temperature overnight with gentle bubbling of argon (Note 4). A 450-watt Hanovia medium-pressure mercury lamp is inserted into the well, through which a fast stream of water is continuously passed (Note 5). The solution is cooled in a dry ice-ethanol bath and stirred vigorously (Note 6). When the temperature of the solution reaches 0°C, the lamp is turned on, and irradiation is continued at 0°C to −20°C for 4 hr (Note 7). The lamp is turned off, a solution of 75 mg (0.34 mmol) of 9-acetylanthracene (Note 8) in 3 mL of tert-BuOMe is added to the solution, and a uranium filter (Note 9) is inserted into the arc housing (Note 10). After 10 min, the lamp

is started again, and the mixture is irradiated at 0°C to −20°C for 1 hr through the uranium filter (Note 11). The cold, pale yellow solution of pre-vitamin **2** thus obtained (Note 12) is transferred to a 3-L, round-bottomed flask and concentrated at 20-25°C under reduced pressure. The residue is transferred again to a 500-mL, round-bottomed flask using tert-BuOMe to allow quantitative transfer. The solution is concentrated and dried at room temperature under high vacuum (1 mm) for 30 min to give approximately 15.4 g of a yellow resin. The flask is filled with argon and equipped with a magnetic stirrer. Methanol (100 mL) is added, and the mixture is shaken to give a stirrable suspension. The suspension is then stirred for 45 min at room temperature and stored in a freezer overnight. After the mixture is stirred at −30°C (Note 13) for 30 min, it is quickly filtered through a 60-mL sintered-glass funnel of coarse porosity. The collected solid is washed with 20 mL of cold methanol (Note 14). Ergosterol (1.83 g, 14% recovery, mp 145-151°C, 99.4% pure) is recovered by washing this solid with absolute ethanol (30 mL at room temperature). The filtrate and cold-methanol wash are transferred to a 500-mL, round-bottomed flask equipped with a magnetic stirrer, an argon-inlet tube, and a reflux condenser. The flask is flushed with argon, and the orange solution is heated under reflux for 6 hr (Note 15) and then stirred at 35-40°C overnight (Note 16). The mixture is concentrated at 30°C under reduced pressure, and the residual methanol is removed by coevaporation with 50 mL of toluene at 30°C to give approximately 14.6 g of an orange-tan oil. The flask is filled with argon and then equipped with a magnetic stirrer. The residue is dissolved in 40 mL of pyridine , and the solution is cooled in an ice-water bath. Solid 3,5-dinitrobenzoyl chloride (9.0 g, 39 mmol) (Note 17) is added in small portions over 5 min followed by 20 mL of pyridine to rinse the walls of the flask, and the mixture is stirred at 0°C for 20 min. The very thick suspension obtained is shaken and then allowed to stand at 0°C for a further 20 min, whereupon methanol (30 mL) is added to the cold mixture. The mixture is allowed to stand at 0°C for 5 min, and then it is shaken for about 5 min to give a stirrable suspension. After the orange suspension is stirred at 0°C for 1.5 hr, it is diluted by the dropwise addition of 150 mL of methanol over 15 min and stirred at 0°C for another hour. The yellow solid is collected by filtration, washed with 50 mL of ice-cold methanol , and dried at room temperature under high vacuum for 2 hr. The yellow-orange solid is transferred to a 250-mL, round-bottomed flask equipped with a magnetic stirrer and an argon-inlet tube. The flask is flushed with argon, and the solid is suspended in 50 mL of absolute ethanol . The suspension is stirred at room temperature for 15 min and at 0°C for 45 min. The solid is collected by filtration, washed with 20 mL of cold methanol , and dried at room temperature under high vacuum overnight to give 8.3-10.1 g (41-50%) of **3** as a yellow solid, mp 139-141°C (lit.[2,3] 147-149°C) (Note 18) and (Note 19).

B. Vitamin D_2 (**4**). A 500-mL, round-bottomed flask equipped with a magnetic stirrer and an argon-inlet tube is charged with 10.1 g (17.1 mmol) of **3** and 171 mL of absolute ethanol . The flask is flushed with argon, and 1.88 mL (18.8 mmol) of 10 N sodium hydroxide is added. After the purple suspension is stirred at room temperature for 45 min, it is cooled with an ice-water bath. Then, 75 mL of ethanol-water (EtOH-H_2O)(2:5) is added over 5 min, and the mixture is stirred for 1 hr with ice-water cooling. To the resulting rose-colored suspension is added 100 mL of EtOH-H_2O (2:5) dropwise over 30 min. The mixture is stirred at 0°C for 30 min and then stored in a refrigerator overnight. The precipitate (Note 12) is collected by filtration using a 60-mL sintered-glass funnel of coarse porosity, washed quickly with 50 mL of cold EtOH-H_2O (3:2) (Note 20), and dried at room temperature under high vacuum to give 6.55 g of an off-white solid. This material is transferred to a 500-mL, round-bottomed flask equipped with a magnetic stirrer and an argon-inlet tube. The flask is flushed with argon, and the solid is dissolved in 200 mL of methanol (MeOH) at room temperature. The solution is cooled with an ice-water bath, and 15 mL of methanol-water (MeOH-H_2O) (4:6) is added to give a cloudy solution. After stirring at 0°C for 1 hr, the resulting white suspension is diluted by the dropwise addition of 85 mL of MeOH=H_2O (4:6) over 45 min. After 2 hr at 0°C, the solid is collected by filtration using a 60-mL sintered-glass funnel of coarse porosity, washed with 50 mL of cold MeOH=H_2O (4:1) (Note 20), and dried at room temperature under high vacuum overnight to give 5.9-6.2 g (87-91%) of **4** as a white solid, mp 112-114°C (lit.[2,3] 114.5-117°C) (Note 21) and (Note 22). The overall yield of vitamin D_2 from ergosterol is 44% (51% based on the recovered ergosterol) (Note 2).

2. Notes

1. The reactor is available from Ace Glass Inc. [reaction vessel (#7851-17), immersion well (#7854-28, 290 mm), Teflon bearing (#8066-24), and stirring shaft (#8068-303)]. It is similar to that shown in

Figure 1 (*Org. Synth., Coll. Vol. V* **1973**, p.529) with an additional stirring chamber. The submitter used a 4-mm I.D. tube for an argon-inlet in order to avoid clogging; the tube should reach near to the bottom of the vessel. The checkers used a 20 × 45-cm (ID × height) Dewar, available from Cole-Parmer Instrument Co. #H-03774-54).

2. Ergosterol (**1**), obtained from Aldrich Chemical Company, Inc. (mp 134-142°C, ε 8,030 at 282 nm in EtOH), was purified before use as follows: 24.4 g of **1** was suspended in 200 mL of ethanol (EtOH), and the mixture was stirred at room temperature for 3 hr prior to filtration. The collected solid was washed with 40 mL of EtOH and dried under high vacuum (1.0 mm) to give 19.3 g (79% recovery) of **1** as a white solid (mp 147-153°C, ε 11,900 at 282 nm in EtOH). The submitter observed that when **1** purchased from Kaneka Co. (mp 147-153°C, ε 11,560 at 282 nm in EtOH) was used as received, a better quality of vitamin D_2 (**4**) (mp 114-115°C, 99.8% pure) was obtained in a better overall yield of 48% (55% based on the recovered ergosterol). The checkers used ergosterol obtained from Acros Organics (mp 156-158°C), which was purified as described above (mp 154-158°C).

3. Ethyl p-dimethylaminobenzoate and tert-butyl methyl ether (HPLC grade) were obtained from Aldrich Chemical Company, Inc. , and used as received.

4. The overnight stirring with bubbling of argon to remove oxygen is probably too long, but was done simply for convenience, thereby allowing the irradiation to be carried out the next day. The checkers found that 4 hr was sufficient.

5. Water flow must be very fast to avoid freezing, which could result in breakage of the photochemical reactor and generation of a hazardous situation. Water flow should be monitored continuously during the course of the reaction.

6. Efficient stirring is necessary to achieve relatively homogeneous temperature distribution throughout the reaction mixture. The submitter recommends that the mixture be kept below 0°C to prevent thermal isomerization of **1** to vitamin D_2, which produces a variety of photoproducts upon irradiation.

7. The checkers monitored the reaction temperature at 5-min intervals and added dry ice to the bath each time the temperature approached 0°C. Approximately 30 pounds of dry ice are required over the 4-hr irradiation period. Using ^{1}H NMR analyses, the submitter judged the conversion to be 80-85% after 4 hr of irradiation with the apparatus described. After 3 hr, a 1:2:1 mixture of **1**:**2**:tachy-isomer (see Discussion) was obtained. The diagnostic peaks in the ^{1}H NMR spectra are listed in Table. The R_f values on silica gel TLC using 1:4 EtOAc-hexane are as follows: **1** (0.29), **4** (0.34), tachy-isomer (0.34), **2** (0.42), ethyl p-dimethylaminobenzoate (0.47), and 9-acetylanthracene (0.53), using short wave UV detection.

TABLE
THE CHEMICAL SHIFTS OF DIAGNOSTIC PEAKS IN ^{1}H NMR[a]

	18-CH_3	3-H	olefinic protons
Ergosterol (**1**)	0.62 (s)	3.62 (m)	5.39 (m), 5.57 (m)
Pre-isomer (**2**)	0.71 (s)	3.92 (m)	5.48 (m), 5.67 (d, 11 Hz), 5.93 (d, 11 Hz)
Tachy-isomer	0.69 (s)	3.92 (m)	5.67 (m), 6.00 (d, 16 Hz), 6.71 (d, 16 Hz)

[a] The chemical shifts are reported in ppm relative to $CHCl_3$ (7.25) in $CDCl_3$ as an internal standard.

8. 9-Acetylanthracene was purchased from Aldrich Chemical Company, Inc. and used as received.

9. A cylindrical uranium filter (31-mm O.D., 2.5-mm thickness) was obtained from Houde Glass Co., Inc.

10. *Caution! The lamp is very hot.* The lamp should be allowed to cool for 10 min before restarting to prevent damage.

11. Based on ^{1}H NMR analyses, the photosensitized isomerization of the tachy-isomer into **2** was complete after 40 min of irradiation (see Discussion).

12. The submitter recommends that pre-isomer (**2**) and vitamin D_2 (**4**) not be exposed to air at room temperature for more than 30 min, since these compounds are relatively easily oxidized by air.

13. The checkers used a dry ice-ethylene glycol bath at −25°C.

14. The checkers cooled the methanol to −70°C in a dry ice-acetone bath.

15. After 3 hr of reflux, the ratio of **2** to **4** was ca. 1:3, whereas after 6 hr, it reached about 1:5.

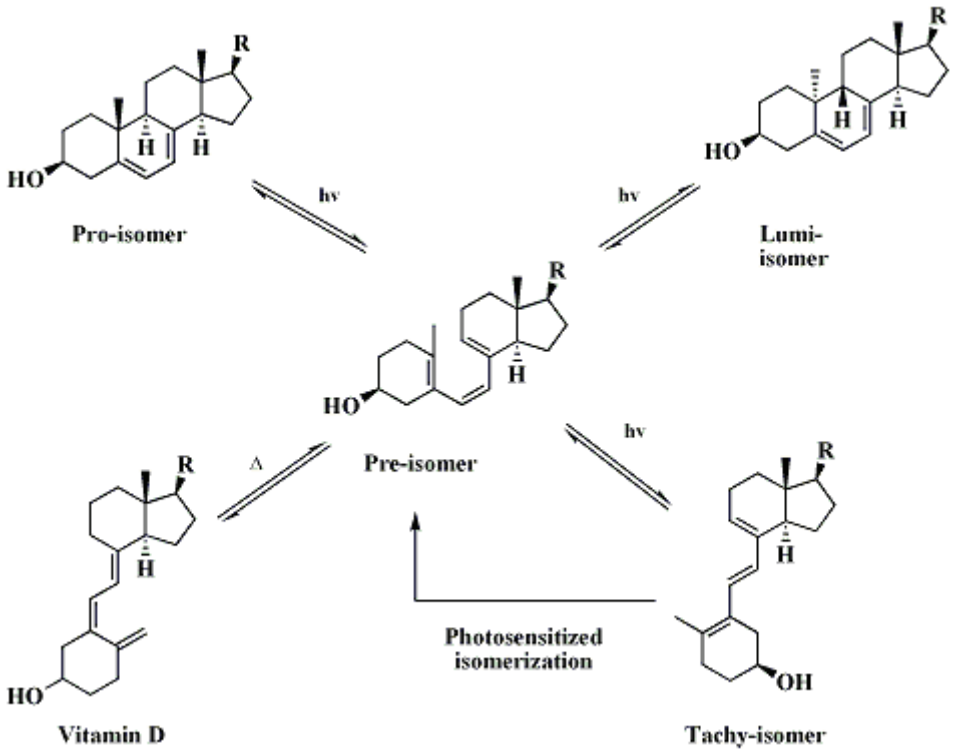
16. At this point, the ratio of **2** to **4** was greater than 1:6.
17. 3,5-Dinitrobenzoyl chloride was purchased from Aldrich Chemical Company, Inc. and pyridine (A.C.S. certified) was obtained from Fisher Scientific Co. They are used as received.
18. The elemental analysis and spectral properties of **3** are as follows: Anal. Calcd for $C_{35}H_{46}N_2O_6$: C, 71.16; H, 7.85; N, 4.74. Found: C, 71.05; H, 7.89; N, 4.58; IR (KBr) cm^{-}: 1733, 1546, 1342 ; ^1H NMR ($CDCl_3$) δ: 0.56 (s, 3 H), 0.82 (d, 3 H, J = 6.5), 0.84 (d, 3 H, J = 6.5), 0.92 (d, 3 H, J = 6.8), 1.02 (d, 3 H, J = 6.6), 1.25-2.17 (m, 16 H), 2.32 (m, 1 H), 2.50 (m, 1 H), 2.59 (dd, 1 H, J = 12.2 and 6.8), 2.73 (dd, 1 H, J = 12.2 and 4.5), 2.80 (dd, 1 H, J = 8.8 and 3.5), 4.91 (bs, 1 H), 5.13 (bs, 1 H), 5.20 (m, 2 H), 5.31 (m, 1 H), 6.06 (d, 1 H, J = 11.1), 6.28 (d, 1 H, J = 11.1), 9.13 (d, 2 H, J = 2.1), 9.22 (t, 1 H, J = 2.1) ; ^{13}C NMR ($CDCl_3$) δ: 12.2 (q), 17.5 (q), 19.5 (q), 19.8 (q), 21.0 (q), 22.1 (t), 23.5 (t), 27.7 (t), 28.9 (t), 31.7 (t), 32.0 (t), 33.0 (d), 40.3 (d and t), 41.9 (t), 42.7 (d), 45.8 (s), 56.3 (d), 74.8 (d), 113.2 (t), 117.1 (d), 122.1 (d), 123.0 (d), 129.3 (d), 131.9 (d), 133.1 (s), 134.3 (s), 135.4 (d), 143.1 (s), 143.8 (s), 148.5 (s), 161.8 (s) (one doublet was not observed).
19. The submitter indicates that HPLC analysis of the product shows its purity to be 97.3% (with 0.3% of ergosteryl 3,5-dinitrobenzoate). HPLC conditions for this unchecked analysis are as follows: column: Chromegasphere SI-60 (3μ,15-cm × 5-mm) (purchased from ES Industries); mobile phase: 5% EtOAc in heptane (1 mL/min); detection: 275 nm. The retention times of **3** and ergosteryl 3,5-dinitrobenzoate are 4.90 and 3.83 min, respectively. Those of the other impurities are 4.20, 4.55, 6.17, and 8.67 min. The checkers observed traces of pyridine in the ^1H NMR spectrum of the product.
20. The checkers cooled this solution in an ice-water bath.
21. The elemental analysis and the spectral properties of **4** are as follows: Anal. Calcd for $C_{28}H_{44}O$: C, 84.79; H, 11.18. Found: C, 84.89; H, 11.17. UV (EtOH) λ_{max} 264 nm (ε 18,450); ^1H NMR ($CDCl_3$) δ: 0.55 (s, 3 H), 0.82 (d, 3 H, J = 6.5), 0.84 (d, 3 H, J = 6.5), 0.91 (d, 3 H, J = 6.5), 1.01 (d, 3 H, J = 6.7), 1.2-2.5 (m, 19 H), 2.57 (dd, 1 H, J = 11.6 and 3.5), 2.81 (broad d, 1 H, J = 9.0), 3.95 (m, 1 H), 4.82 (bs, 1 H), 5.04 (bs, 1 H), 5.20 (m, 2 H), 6.05 (d, 1 H, J = 11.2), 6.23 (d, 1 H, J = 11.2) ; The OH hydrogen was not observed; ^{13}C NMR ($CDCl_3$) δ: 12.2 (q), 17.5 (q), 19.5 (q), 19.8 (q), 21.0 (q), 22.1 (t), 23.4 (t), 27.7 (t), 28.9 (t), 31.8 (t), 33.0 (d), 35.0 (t), 40.3 (d and t), 42.7 (d), 45.6 (s), 45.8 (t), 56.3 (d), 69.1 (d), 112.3 (t), 117.4 (d), 122.3 (d), 131.8 (d), 134.9 (s), 135.5 (d), 142.1 (s), 144.0 (s) (one doublet was not observed).
22. The submitter indicates that HPLC analysis of the product shows the purity to be 99.7% (with 0.1% of ergosterol). The HPLC conditions for this unchecked analysis are as follows: column: Chromegasphere SI-60 (3μ, 15-cm × 5-mm) (purchased from ES Industries); mobile phase: 5% EtOAc in heptane (2 mL/min); detection: 275 nm. The retention times of **1** and **4** are 19.54 and 16.27 min, respectively. The checkers observed signals due to a trace olefinic contaminant in the ^1H NMR of the product at δ 6.47 and 5.95.

Waste Disposal Information

All toxic materials were disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995.

3. Discussion

The overall yields of vitamin D derivatives[4 5] via the photolyses of pro-isomers (such as **1**) are, generally only 15-30% (typically on a few milligram scale). Difficulties in obtaining higher yields arise from the fact that the photolysis of the pro-isomer gives a photostationary state where the distribution of the products (pro-, pre-, lumi-, and tachy-isomers) depends on the photolyzing wavelength[6 7 8 9] (see Scheme). A shorter wavelength (<290 nm) leads to a predominance of the ring-opened products (pre- and tachy-isomers), and a longer wavelength (>300 nm) promotes the ring-closure reaction to the pro- and lumi-isomers. Furthermore, the pre-isomer and vitamin D are in thermal equilibrium in which the ratio is dependent on the temperature (Scheme).[10 11]



Higher yields of the pre-isomer have been achieved in the past by irradiating the pro-isomer with a narrow band of approximately 250 nm light (using a low pressure mercury lamp or a laser) and then selective isomerization of the tachy-isomer thus formed into pre-isomer, either by irradiation at approximately 350 nm[7,8] or by photosensitized isomerization.[12 13] Vitamin D_3 was then isolated in 50% yield after thermal isomerization.[8] However, the use of high-intensity light sources with narrow band spectra, for large scale applications is prohibitive because of their high cost; moreover, such sources require the use of specialized equipment.

The present procedure uses a medium pressure mercury lamp, which is an inexpensive, high-intensity light source commonly used in a synthetic laboratory. The 300-315 nm light that promotes the ring-closure reaction is effectively removed by adding a small amount of ethyl p-dimethylaminobenzoate which has a strong, relatively sharp absorption at 305 nm (ε 32,500). After the photosensitized isomerization and thermal equilibration, vitamin D_2 is isolated as the 3,5-dinitrobenzoate **3**.[2,3] Hydrolysis of this relatively stable derivative furnishes crystalline vitamin D_2 in high purity. This procedure, which uses readily available and inexpensive photolysis equipment, proceeds in good yields and does not require chromatographic purification of the product.

References and Notes

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Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

Vitamin D_2: Ergocalciferol (8);
9,10-Secoergosta-5,7,10 (19),
22-tetraen-3-ol, (3β)- (9); (50-14-6)

Ergosterol (8);
Ergosta-5,7,22-trien-3-ol, (3β)- (9); (57-87-4)

Vitamin D_2 3,5-dinitrobenzoate:
Ergocalciferol, 3,5-dinitrobenzoate (8,9); (4712-11-2)

Ethyl p-dimethylaminobenzoate:
Benzoic acid, p-(dimethylamino)-, ethyl ester (8);
Benzoic acid, 4-(dimethylamino)-, ethyl ester (9); (10287-53-3)

tert-Butyl methyl ether:
Ether, tert-butyl methyl (8);
Propane, 2-methoxy-2-methyl- (9); (1634-04-4)

9-Acetylanthracene:
Ketone, 9-anthryl methyl (8);
Ethanone, 1-(9-anthracenyl)- (9); (784-04-3)

3,5-Dinitrobenzoyl chloride:
Benzoyl chloride, 3,5-dinitro- (8,9); (99-33-2)