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A New Synthesis of Benzo[*a*]pyrene. 7,10-Dimethylbenzo[*a*]pyrene[1]

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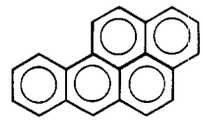
1-Acetylpyrene was reacted with the lithium enolate of ethyl acetate to yield the hydroxy ester which was dehydrated to 3-(1-pyrenyl)-2-butenoic acid. Reduction produced 3-(1-pyrenyl)butanoic acid which was reduced by $LiAlH_4$ to 3-(1-pyrenyl)butanol. Mesylation, cyanation, and hydrolysis afforded 4-(1-pyrenyl)pentanoic acid. This acid was produced in 15% yield but in one step by alkylation of pyrene with γ-valerolactone. Cyclization with HF produced 7-keto-10-methyl-7,8,9,10-tetrahydrobenzo[*a*]pyrene from which 10-methylbenzo[*a*]pyrene was produced by reduction and dehydrogenation and 9,10-dimethylbenzo[*a*]pyrene by reaction with methyllithium followed by dehydration and dehydrogenation. Alternately 7,10-dimethylbenzo[*a*]pyrene was synthesized by reaction of 1-bromopyrene with 2,5-dimethylfuran (via 1-pyryne) to yield 7,10-dihydro-7,10-dimethyl-7,10-epoxybenzo[*a*]-pyrene which on reduction and acid-catalyzed dehydration yielded 7,10-dimethylbenzo[*a*]pyrene. The fact that 10-methylbenzo[*a*]pyrene is inactive as a carcinogen is discussed in terms of the effect of the 10-methyl group on the metabolism involved.

The metabolism of benzo[*a*]pyrene, **1**, in relation to carcinogenicity and mutagenicity has long interested scientists. Recently, the hypothesis has been advanced that one (or more) of the isomeric 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenes, **2**, is the ultimate carcinogen, and/or mutagen.[3] Presumably, epoxidation[4] of **1** to yield the 7,8-



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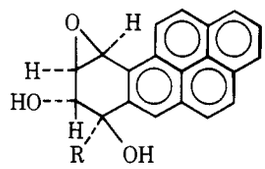


2, R = H
4, R = CH_3

epoxide occurs first, followed by hydration to trans-7,8-diol which is then epoxized to **2**. Thus three enzyme-catalyzed processes occur consecutively to yield (presumably) the ultimate carcinogenic species.

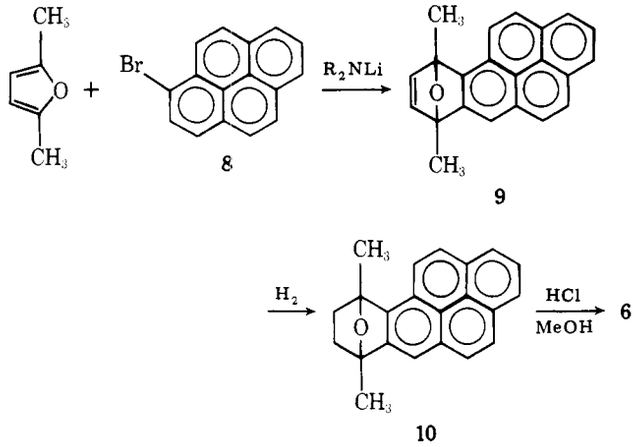
In studies in the benz[*a*]anthracene series, we have hypothesized that the carcinogenic activity of many substituted benz[*a*]anthracenes can be rationalized if it is assumed that: (a) detoxification occurs by metabolic attack at position 7; and (b) carcinogenic activity arises by metabolism which is initiated by attack at position 5.[5,6] Each of these metabolic pathways can be blocked (or altered) by substitution of a methyl or fluoro group at the position involved. For example, 7,12-dimethylbenz[*a*]anthracene is a potent carcinogen but 5,7,12-trimethylbenz[*a*]anthracene[7] and 7,12-dimethyl-5-fluorobenz[*a*]anthracene[7,8] are inactive, probably because in each the 5 position is blocked.

Because of the results in the benz[*a*]anthracene series we became interested to find out if the current hypothesis concerning the ultimate carcinogen in the benzo[*a*]pyrene series could be tested by determining the carcinogenic activity of benzo[*a*]pyrenes having methyl groups in the benz ring.

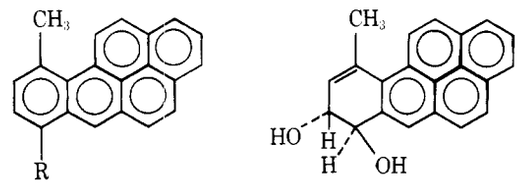
For example, if a methyl group were substituted at the 7 position of benzo[*a*]pyrene would this block the epoxidation at the 7,8 position and render the compound inactive? Since 7-methylbenzo[*a*]pyrene, **3**, has been reported to be carcinogenic,[9] evidently the epoxidation at the 7–8 bond can occur as well as the hydration of the 7,8-oxide to the 7-methyl-7,8-*trans*-diol. The latter could then be epoxidized at the 9–10 bond to yield a 7,8-dihydroxy-9,10-epoxy-7-methyl-7,8,9,10-tetrahydrobenzo[*a*]pyrene, **4**, which would be a corcinogenic substance analogous to the product, **2**, formed from **1**. The above reasoning assumes that the carcinogenic activity of **3** is due to the same type of metabolic processes responsible for the activation of **1**.

Accordingly, we wished to know if 10-methylbenzo[*a*]pyrene, **5**, and 7,10-dimethylbenzo[*a*]pyrene, **6**, would be carcinogenic. We have been informed that after 16 months, **5** has produced no tumors and hence must be considered inactive as a carcinogen.[7] One can assume that **5** is capable of being epoxidized and the epoxide hydrated to *trans*-7,8-dihydro-7,8-dihydroxy-10-methylbenzo[*a*]pyrene, **7**, but epoxidation at the 9,10 position is either blocked or the epoxide, if formed, does not interact with DNA as does **2**. Further experiments

Scheme I



to sort out the possibilities are in order. We will be glad to supply quantities of **5** and **6** to interested researchers. Tests

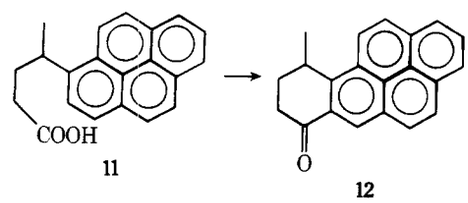


5, R = H
6, R = CH_3

on **6** have been initiated[7] but it is too early to assess its activity. Presumably it will prove inactive because of the blocking effect of the methyl group at the 10 position.

7,10-Dimethylbenzo[a]pyrene, **6**, was synthesized by a route (Scheme I) involving condensation of 1,2-pyryne with 2,5-dimethylfuran. A similar condensation with furan failed because of metallation.[11]

Improvements in the synthesis of **5**[10] included a one-step, time-saving condensation of pyrene with γ-valerolactone[12] to form **11**, a route superior to others despite the 15% yield.



Cyclization of **11** to 7-keto-10-methyl-7,8,9,10-tetrahydrobenzo[a]pyrene, **12**, followed by conventional chemistry led to the synthesis of both **5** and **6**.

Experimental Section[13]

4-(1-Pyrenyl)pentanoic Acid, 11. This compound was prepared essentially as described[10] from 1-acetylpyrene except that the homologation of 3-(1-pyrenyl)-butanoic acid was accomplished by reduction to 3-(1-pyrenyl)butanol (*m/e* 274)[14] with $LiAlH_4$ in benzene–ether (reflux, 6 h) in almost quantitative yield (single spot on TLC) followed by conversion (almost quantitatively) to the mesylate (*m/e* 352)[14] by treatment with mesyl chloride in CH_2Cl_2–$(C_2H_5)_3N$ at 0 °C for 75 min. To a solution of 37.1 g (0.1 mol) of mesylate and 1.5 g of Aliquat 336[15] in 150 mL of benzene was added a solution of 40 g of KCN in 100 mL of water. The mixture was refluxed and well stirred for 48 h. After removing solvent from the washed benzene layer, a solution of the residue (single spot on TLC different from mesylate) in 100 mL of ethanol and 100 mL of 40% KOH was refluxed for 20 h. Since the crude acid, **11**, produced melted at 126–128 °C and after recrystallization from benzene–acetic acid at 129.5–130.5 °C (lit.[10] mp 135–136 °C of purified **11**) evidently a polymorphic form was at hand. The overall yield from 3-(1-pyrenyl)butanoic acid was 80.7%. Our procedure represents an improvement of the literature method[10] in that larger amounts of material can be more easily processed and the overall yield of pure acid is better.

In another synthesis of **11**, 16.2 g of $AlCl_3$ was added during 5–10 min to a well-stirred mixture of 20.2 g of commercial pyrene[16] and 100 mL of *o*-dichlorobenzene followed by 8.0 g of freshly distilled γ-valerolactone. The reaction mixture was gradually heated to 50–55 °C, held there for 48 h, and poured after cooling into ice–HCl. After removal of solvent by steam distillation an ether solution of the products was extracted with water and then 3% NaOH. Acidification of the alkaline extract gave 8 g of crude **11**. The methyl ester was prepared, distilled, and chromatographed over silica gel to yield 4.2 g of methyl ester (single spot on TLC). Hydrolysis and recrystallization of the acid from benzene–petroleum ether, 30–60 °C, afforded 3.7 g of colorless **11**, mp 130–131 °C (lit.[10] mp 135–136 °C, a polymorphic form as we never got a melting point higher than 130–131 °C). An overall yield of 15.3% based on valerolactone was obtained. The yield of pure **11** was smaller if the Friedel–Crafts reaction was run at 120 °C for 4 h.

3-(1-Pyrenyl)butanoic Acid. To an ether solution of 0.22 mol of methyllithium (2 M) was added 30.8 g of 2,2,6,6-tetramethylpiperidine[17] followed after 5 min at 0 °C with 100 mL of pure THF. To this solution at −78 °C was added 19.3 g of ethyl acetate[18] during 5 min followed after 15 min with a solution of 50 g (0.21 mol) of 1-acetylpyrene[10] in 250 mL of THF added during 20 min. After 45 min at −78 °C the reaction mixture was treated with dilute HCl. A conventional workup yielded crude hydroxy ester which was heated with 350 mL of toluene and 0.5 g of toluenesulfonic acid for 5 h, the water formed being removed by distillation. Alkaline hydrolysis afforded 44 g (89% based on recovery of 8 g (16%) of 1-acetylpyrene) of 3-(1-pyrenyl)-2-butenoic acid, mp 228–232 °C (lit.[19] mp 233 °C). Catalytic hydrogenation of 4 g of acid in 40 mL of THF over 150 mg of PtO_2 (Engelhard) for 4 h at 50 psi afforded 3.2 g (80%) of 3-(1-pyrenyl)butanoic acid, mp 177–178 °C (lit.[10] mp 177–178 °C), after crystallization from benzene.

7-Keto-10-methyl-7,8,9,10-tetryhydrobenzo[a]pyrene, 12. To 250 mL of HF in a polyethylene bottle was added 24.0 g of **11** with stirring. After 30 min the HF was evaporated in a rapid stream of N_2 and the residue was treated with aqueous $NaHCO_3$ and filtered. The neutral product was recrystallized from benzene–petroleum ether to yield 15.2 g of **12**, mp 154–159 °C (combined yield 97%). Recrystallization afforded pure **12** mp 162–163 °C (lit.[10] mp 162–163 °C) with little loss. More than 30 min contact with HF gave lower yields.

10-Methylbenzo[a]pyrene, 5. The conversion of **12** to **5**, mp 188–190 °C, was accomplished essentially as described[10] in 41% overall yield. When the aromatization of the secondary alcohol was effected by heating at 300–360 °C for 30 min over 5% rhodium-on-alumina[20] the yield was 51%. Pure **5**, mp 192–193 °C (lit.[10] mp 190–190.8 °C), was obtained with little loss by vacuum sublimation.

7,10-Dimethylbenzo[a]pyrene, 6. A solution of lithio *N*-cyclohexylisopropylamine prepared by treating 3.5 g (0.025 mol) of amine in 50 mL of THF with an equivalent of butyllithium in hexane was added over 15 min to a stirred solution of 6.7 g of 1-bromopyrene[21] and 11.5 g of 2,5-dimethylfuran in 100 mL of THF at room temperature. After refluxing for 4 h the mixture was poured on ice–HCl. A benzene–ether extract of the products was washed with water and saturated NaCl solution and dried by passing over $MgSO_4$. After removal of solvent the residue was triturated with 30–60 °C petroleum ether. The solid obtained (1.4 g) contained nitrogen and was discarded. Recrystallization of the material from the filtrate yielded 1.1 g of **9**, mp 167–168 °C. Column chromatography of the material from the mother liquors afforded first 1.5 g of pyrene and then 1.2 g of **9** (total yield 33%): mp 167–168 °C; *m/e* 296, NMR ($CDCl_3$, $(CH_3)_4Si$, ppm) 127 (s, 3, CH_3), 144 (s, 3 H, CH_3), 413–441 (m, 2 H, CH=CH), 467–504 (m, 8 H, ArH).[22] Anal. Calcd for $C_{22}H_{16}O$: C, 89.1; H, 5.4. Found:[23] C, 88.8, H, 5.4.

About the same yield was obtained when 2,2,6,6-tetramethylpiperidine[17] was used in place of cyclohexylisopropylamine. This reaction failed when run in glyme or when lithium hexamethyl disilazane was used in THF.

A solution of 0.80 g of **9** in 30 mL of THF was reduced over PtO_2 at 15 psi for 1 h to yield a yellow solid (*m/e* 298) which was suspended in 50 mL of methanol saturated with HCl. After heating at reflux for 2.5 h the solvent was removed under reduced pressure and the residue was triturated with saturated Na_2CO_3 and filtered. Recrystallization of the solid from acetic acid yielded crude **6** in 87% yield. Sublimation afforded pure **6** as a bright yellow solid: mp 167–168 °C with little loss; *m/e* 280; NMR ($CDCl_3$) 176 (s, 3, $ArCH_3$), 196 (s, 3, $ArCH_3$), 434–568 ppm (m, 10, ArH). Anal. Calcd for $C_{22}H_{16}$: C, 94.1; H, 5.8. Found:[23] C, 94.3; H, 5.7.

Alternately, a sample of **6** was prepared by reaction of **12** with

methyllithium in ether–benzene, followed by dehydration of the tertiary alcohol formed and aromatization at 300–330 °C for 30 min over Rh-on-Al_2O_3.[20] The melting point and mixture melting point of **6** prepared by the two routes was 167–168 °C.

Registry No.—**1**, 50-32-8; **5**, 63104-32-5; **6**, 63104-33-6; **8**, 1714-29-0; **9**, 63104-34-7; **11**, 63104-35-8; **12**, 63104-36-9; 3-(1-pyrenyl)butanol, 63104-37-0; 3-(1-pyrenyl)butanol mesylate, 63104-38-1; mesyl chloride, 124-63-0; 3-(1-pyrenyl)butanoic acid, 63104-39-2; ethyl acetate, 141-78-6; 1-acetylpyrene, 3264-21-9; 3-(1-pyrenyl)-2-butenoic acid, 63104-40-5; 2,5-dimethylfuran, 625-86-5.

References and Notes

(1) This research was supported by Contract CP 43248 and Grant CA-07394 from the National Cancer Institute of The National Institutes of Health.
(2) Postdoctoral Research Associate.
(3) See (a) A. M. Jeffrey, R. G. Harvey, K. Nakanishi, et al., *J. Am. Chem. Soc.*, **98**, 5714 (1976); (b) M. Koreeda, D. M. Jerina, et al., *ibid.*, **98**, 6720 (1976), for pertinent literature references and discussion.
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(7) Unpublished experiments by James A. and Betty C. Miller, McArdle Laboratory for Cancer Research, University of Wisconsin.
(8) Unpublished experiments by Charles Huggins, Ben May Laboratory for Cancer Research, University of Chicago.
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(13) All mp and bp are uncorrected and in °C. All anhydrous ether was distilled from Grignard reagent and THF from $LiAlH_4$. All chromatography was carried out with distilled solvents.
(14) We thank Mr. R. Weisenberger for the mass spectral determinations.
(15) Methyltricapyrlammonium chloride obtained from the McKerson Corp., 3016 Fourth Ave. S., Minneapolis, Minn.
(16) Aldrich Chemical Co., Milwaukee, Wis. 53233.
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(18) Procedure essentially that of M. W. Rathke, *J. Am. Chem. Soc.*, **92**, 3222 (1970).
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(22) Alternately, the purification could be accomplished more conveniently by dry column chromatography over neutral Woelm alumina using benzene–petroleum ether, bp 30–60 °C (1:1), as described by B. Loev and M. M. Goodman, *Chem. Ind.*, 2026 (1967).
(23) Microanalyses by M-H-W Laboratories Garden City, Mich. 48135.

Isonicotinyloxycarbonyl, a Novel Amino Protecting Group for Peptide Synthesis[1]

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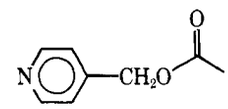
The isonicotinyloxycarbonyl (iNoc) group is an acid-stable protecting group for the protection of the ϵ-amino group of lysine. iNoc can be removed by reduction with Zn under mild acidic conditions or by catalytic hydrogenation. The combined physical and chemical properties of iNoc offer unique advantages for its use as a lysine protecting group.

We[2] and others[3,4] have observed undesired loss of the benzyloxycarbonyl protecting group used for the protection of the ϵ-amino group of lysine during acid-catalyzed removal of the α-amine protecting group, *tert*-butyloxycarbonyl. Methods for more selective removal of the *tert*-butyloxycarbonyl group in the presence of benzyloxycarbonyl have been proposed.[5] Protecting groups for lysine of greater acid stability[6] or more acid labile α-amino protecting groups[7] have been used to avoid these problems. The former approach has been successfully used in solid-phase peptide synthesis.[6] For a variety of reasons it is less satisfactory for synthesis in solution.[8] These improvements still rely on kinetic differences in the rates of removal of two acid labile protecting groups cleaved by different mechanisms. Cleavage of the more labile protecting groups generally proceeds predominantly via an S_N1 pathway, while the more stable protecting groups follow an S_N2 pathway. Thus, modifications in removal conditions by the introduction of nucleophilic scavengers or solvent changes may result in a loss of selectivity. We have noted such decreased selectivity for removal of the *tert*-butyloxycarbonyl group in the presence of the benzyloxycarbonyl group.[9] For this reason, we preferred an ϵ-amino lysine protecting group which is completely stable to acid, but which can be smoothly removed under mild conditions, for instance, reductively. Such a protecting group would assure absolute stability when the *tert*-butyloxycarbonyl is cleaved with acid. Conversely, such an ϵ-amino protecting group could be removed reductively without affecting a butyloxycarbonyl group.

Although kinetic selectivity for protecting group removal has, for example, been successfully applied in a synthesis of human insulin,[10] there are advantages to tactics based on a choice of protecting groups removed by chemically different methods. The known protecting groups which would offer chemical selectivity do not fulfill all of our other requirements for a lysine protecting group.[11] We have discussed our criteria in detail elsewhere.[8] These include (1) stability under conditions employed in peptide synthesis, (2) removal under unique and mild chemical conditions, (3) stability under conditions for the removal of other protecting groups, and (4) capability to increase rather than decrease the solubility of large peptides in polar solvents.

The isonicotinyloxycarbonyl protecting group (1) appeared



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to offer the desired combination of properties to meet these criteria. The pyridine ring should make 1 highly stable under acidic conditions, while facilitating reductive removal, as was the case for the carboxyl protecting, 4-picolyl esters of Young.[12]

Isonicotinyl *p*-nitrophenyl carbonate (**3b**) was prepared by the reaction of bis(nitrophenyl) carbonate (**2b**) with 4-hydroxymethylpyridine in the presence of *N*-methylmorpholine