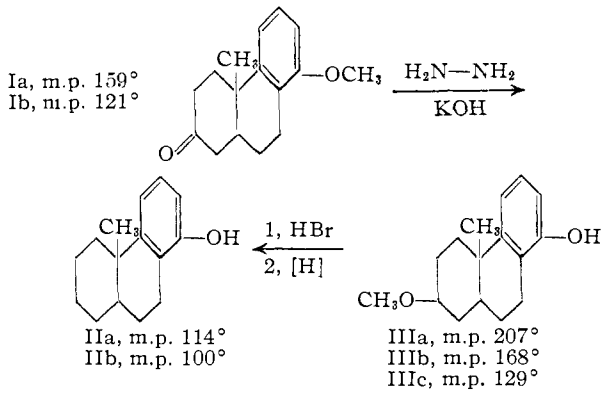

have characterized the 4a-methyl-1,2,3,4,4a,9,10,-10a-octahydro-8-phenanthrols (II). We have prepared these compounds by Huang-Minlon reduction[2] and demethylation of the methoxy ketones Ia (*trans*) and Ib (*cis*), and also by cleavage of the ether groups in the methoxyphenanthrols IIIa (*trans*), IIIb (*cis*) and IIIc (*cis*) with excess hydrobromic acid followed by dehalogenation[3] with Raney nickel alloy. The former method is more satisfactory because treatment of III with excess, concentrated hydrobromic acid leads to considerable resinification. The isomer IIa was obtained from both Ia and IIIa and must have the *trans* arrangement of the alicyclic rings. The isomer IIb was obtained from Ib, IIIb and IIIc and must have the *cis* configuration.

The phenylurethans were found to be good derivatives for further characterizing IIa and IIb, and also good derivatives for IIIa, IIIb and IIIc.

Experimental

Method A.—The methoxy ketone (Ia or Ib, 340 mg.), potassium hydroxide (270 mg.), 95% hydrazine (0.2 ml.) and diethylene glycol (2 ml.) were heated and stirred until homogeneous. A short air condenser was attached and the solution heated under nitrogen at 175–185° for 90 min., then at 195° for 2 hours. An additional 250 mg. of potassium hydroxide was added and the temperature maintained at 225° for 5 hours. The reaction mixture was diluted with water and acidified. The resulting brown solid was collected on a filter and purified by distillation at about 1 mm. followed by crystallization from ligroin; yield 70–75%.

***dl*-4a-Methyl-1,2,3,4,4a,9,10,10aα-octahydro-8-phenanthrol (IIa)**, clear, irregular plates, m.p. 114°. *Anal.* Calcd. for $C_{15}H_{20}O$: C, 83.29; H, 9.32. Found: C, 83.23; H, 9.32.

The phenylurethan was prepared by heating the phenanthrol (60 mg.) with dry pyridine (2 drops) and phenyl isocyanate (4 drops) at 90° for 1 hour. Pyridine and excess phenyl isocyanate were removed by distillation at reduced pressure and the residue crystallized from ligroin; small, well-formed needles, m.p. 126–127°. *Anal.* Calcd. for $C_{22}H_{25}O_2N$: C, 78.77; H, 7.51. Found: C, 78.51; H, 7.41.

***dl*-4a-Methyl-1,2,3,4,4a,9,10,10aβ-octahydro-8-phenanthrol (IIb)**, irregular fragments, m.p. 99–100°. *Anal.* Calcd. for $C_{15}H_{20}O$: C, 83.29; H, 9.32. Found: C, 83.12; H, 9.21.

The phenylurethan, clear, irregular fragments, m.p. 147–148°. *Anal.* Calcd. for $C_{22}H_{25}O_2N$: C, 78.77; H, 7.51. Found: C, 78.47; H, 7.51.

Method B.—The methoxyphenanthrol (IIIa, IIIb or IIIc, 500 mg.) and 48% hydrobromic acid (3 ml.) were warmed to about 100° and acetic anhydride (50 drops) added cautiously. Sufficient acetic acid was added to produce a homogeneous solution and the mixture heated at 110° for 2 hours. Excess hydrobromic and acetic acids was distilled at reduced pressure, the residue washed with water and taken up in 10% sodium hydroxide. The alkaline solution was heated in a boiling water-bath and treated with Raney nickel alloy (1.5 g.). The nickel was filtered off, washed with alcoholic alkali and the filtrate acidified. The precipitated phenanthrol was extracted with ether, washed, distilled at about 1 mm. and crystallized from ligroin; yield 10–20%. The product obtained from IIIa was identical with the material obtained by method A from Ia, and the product from both IIIb and IIIc was identical with that from Ib.

The melting range of an intimate mixture of IIa and IIb was 87–96°.

The phenylurethan of *dl*-2α-methoxy-4a-methyl-1,2,3,4,-4a,9,10,10aα-octahydro-8-phenanthrol (IIIa), fine needles, m.p. 160–161°. *Anal.* Calcd. for $C_{23}H_{27}O_3N$: C, 75.60; H, 7.45. Found: C, 75.51; H, 7.55.

The phenylurethan of *dl*-2β-methoxy-4a-methyl-1,2,3,4,-4a,9,10,10aβ-octahydro-8-phenanthrol (IIIb), very fine needles from benzene–ligroin, m.p. 190–192°. *Anal.* Calcd. for $C_{23}H_{27}O_3N$: C, 75.60; H, 7.45. Found: C, 75.63; H, 7.72.

The phenylurethan of *dl*-2α-methoxy-4a-methyl-1,2,3,4,-4a,9,10,10aβ-octahydro-8-phenanthrol (IIIb), fine needles, m.p. 179–180°. *Anal.* Calcd. for $C_{23}H_{27}O_3N$: C, 75.60; H, 7.45. Found: C, 75.40; H, 7.38.

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7-Alkyl Derivatives of 2-Aminofluorene[1]

BY EUGENE SAWICKI

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4-Methylaminoazobenzene[2] and 2-aminofluorene[3] are carcinogens. A methyl group in the extended *para* position of 4-methylaminoazobenzene causes a marked decrease in carcinogenic activity[4] while an ethyl group in the analogous position causes a slight increase in activity[5] as compared to 4-methylaminoazobenzene.

Assuming that approximately the same forces are operative as in the azo dyes one could expect 7-methyl-2-aminofluorene to be, at the most, weakly carcinogenic, while the 7-ethyl analog could be strongly carcinogenic. On the other hand, any differences in activity among the analogous derivatives of the diverse groups of carcinogens would signify an important difference in chemical or physical reactivity at that level.

The nitration of 2-methyl- and 2-ethylfluorene gave a mononitro-7-methylfluorene and a mononitro-7-ethylfluorene whose almost identical ultraviolet spectra are very similar to that of 2-nitrofluorene, Table I. Comparison of the absorption spectra of the derived acetylamino-7-methylfluorene and acetylamino-7-ethylfluorene with 1-, 2- and 4-acetylaminofluorene[6,7] in Table I shows a definite

(1) The investigation was supported by research grant C-1308 from the National Cancer Institutes of the National Institutes of Health, U. S. Public Health Service.

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TABLE I

	λ_{min} (log ϵ)	λ_{max} (log ϵ)			
2-NF[a]	223 (3.94)	233 (3.98)	331 (4.26)		
	265 (3.22)				
7-Methyl-2-NF	223 (3.94)	238 (4.04)	342 (4.28)		
	270 (3.23)				
7-Ethyl-2-NF	223 (3.94)	238 (4.04)	342 (4.30)		
	270 (3.23)				
2-AAF[b]	240 (3.30)	282[c] (4.41)	288 (4.46)	301[c] (4.25)	315 (4.14)
7-Methyl-2-AAF	242 (3.31)	283[c] (4.43)	290 (4.46)	305[c] (4.25)	316 (4.14)
7-Ethyl-2-AAF	242 (3.30)	283[c] (4.43)	290 (4.47)	305[c] (4.27)	316 (4.17)
1-AAF[d]	233 (4.1)		250 (4.3)	290 (3.72)	302 (3.68)
			265 (4.3)		
4-AAF[d]	234 (2.94)		264 (4.3)	287 (3.94)	298 (3.90)

[a] NF = nitrofluorene. [b] AAF = acetylaminofluorene. [c] Shoulder. [d] Values have been taken from a spectral curve.

spectral similarity between the alkyl derivatives and 2-acetylaminofluorene.

The oxidation of nitro-2-ethylfluorene gave two yellow compounds. One was identified as 2-nitro-7-acetylfluorene by a mixed melting point with an authentic sample.[8] The other compound was 2-nitro-7-ethylfluorenone.

From these facts one can conclude that the nitration of 2-methyl- and 2-ethylfluorene takes place in the 7-position.

Experimental[9]

7-Methyl-2-nitrofluorene.—Concentrated nitric acid (2.4 ml., d. 1.42) was added in one batch to a stirred solution of 1.95 g. of 2-methylfluorene[10] in 12 ml. of acetic acid at 60–65°. The solidified mixture was warmed to 80° and allowed to cool. The product was filtered and washed with a little acetic acid. Yellow microcrystals (2.15 g., 96%) were obtained, m.p. 179–180°. Crystallization from heptane gave yellow needles, m.p. 180–181°. Methyl cellosolve or acetic acid can also be used as crystallizing solvents.

Anal. Calcd. for $C_{14}H_{11}NO_2$: C, 74.7; H, 4.89; N, 6.22. Found: C, 75.0; H, 4.91; N, 6.40.

7-Methyl-2-aminofluorene.—To a suspension of 2.4 g. of 7-methyl-2-nitrofluorene in 60 ml. of boiling alcohol was added a solution of 0.7 g. of calcium chloride in 14 ml. of water and 21 g. of zinc dust. The mixture was vigorously refluxed for 3 hours and then filtered hot. The residue was extracted with alcohol. The filtrates were added to excess water. Colorless crystals (2.0 g., 96%) were obtained, m.p. 103–105°. Crystallization from aqueous alcohol gave glistening colorless needles, m.p. 105–106°.

Anal. Calcd. for $C_{14}H_{13}N$: N, 7.18. Found: N, 7.21.

7-Methyl-2-acetylaminofluorene.—Acetic anhydride (1.0 ml.) was added dropwise to a hot solution of 1.95 g. of 7-methyl-2-aminofluorene in 28 ml. of benzene. The mixture was refluxed half an hour and allowed to cool. The product (2.15 g.) was crystallized from benzene to give 2.0 g. (84%) of glistening needles, m.p. 199–200°.

Anal. Calcd. for $C_{16}H_{15}NO$: C, 81.0; H, 6.33; N, 5.91. Found: C, 80.9; H, 6.53; N, 5.99.

7-Methyl-2-trifluoroacetylaminofluorene.—This compound was prepared by the reaction between trifluoroacetic anhydride and a benzene solution of 7-methyl-2-aminofluorene. Crystallization from heptane gave a 94% yield of colorless needles, m.p. 225°.

Anal. Calcd. for $C_{16}H_{12}F_3NO$: C, 66.0; H, 4.12. Found: C, 66.4; H, 4.05.

2-Ethylfluorene was prepared and purified by the procedure of Campbell and Wang.[11] It melted at 99.5–100.5°; lit. m.p. 81–82°.[11] The compound has a bright blue fluorescence in solution. Oxidation of 2-ethylfluorene, m.p. 99.5–100.5°, by Campbell and Wang's procedure gave 2-ethylfluorenone, m.p. 127–128°, and 2-acetylfluorenone, m.p. 156–157°. Campbell and Wang reported the melting points as 127–128° and 156–157°.

7-Ethyl-2-nitrofluorene.—2-Ethylfluorene was nitrated by the procedure used for the methyl analog. An 85% yield of light yellow plates was obtained, m.p. 158–159°. The pure compound was obtained from heptane in light yellow feathery crystals, m.p. 159–160°.

Anal. Calcd. for $C_{15}H_{13}NO_2$: C, 75.3; H, 5.44; N, 5.86. Found: C, 75.1; H, 5.54; N, 5.80.

2-Nitro-7-ethylfluorenone and 2-Nitro-7-acetylfluorene.—Powdered chromium trioxide (1.2 g.) was sprinkled into a boiling solution of 1 g. of 2-nitro-7-ethylfluorene in 10 ml. of acetic acid. The mixture was refluxed an additional hour and then poured into dilute sulfuric acid. The yellow precipitate was dissolved in benzene and chromatographed on Alumina. The lower yellow layer gave yellow needles of 2-nitro-7-ethylfluorenone, m.p. 169–170° (heptane).

Anal. Calcd. for $C_{15}H_{11}NO_3$: C, 71.1; H, 4.35. Found: C, 71.0; H, 4.40.

The upper yellow layer gave yellow crystals of 2-nitro-7-acetylfluorene, m.p. 227–228° (acetic acid). The mixed melting point with an authentic sample,[8] m.p. 230–231°, was 228–231°. The infrared spectra were identical.

7-Ethyl-2-aminofluorene.—7-Ethyl-2-nitrofluorene was reduced by the procedure used for the methyl analog. An 86% yield of colorless microcrystals was obtained, m.p. 117–118°. Crystallization from hexane gave colorless needles, m.p. 117.5–118.0°.

Anal. Calcd. for $C_{15}H_{15}N$: N, 6.7. Found: N, 6.50.

7-Ethyl-2-acetylaminofluorene.—The acetylation of 7-ethyl-2-aminofluorene in benzene solution with acetic anhydride gave the acetylated derivative in 90% yield, m.p. 185–186°. Crystallization from heptane gave colorless needles, m.p. 185–186°.

Anal. Calcd. for $C_{17}H_{17}NO$: C, 81.3; H, 6.77; N, 5.58. Found: C, 81.5; H, 6.86; N, 5.66.

7-Ethyl-2-trifluoroacetylaminofluorene.—The acylation of 7-ethyl-2-aminofluorene in benzene solution with trifluoroacetic anhydride gave a 92% yield of the fluorine derivative in colorless, gleaming crystals, m.p. 209–210°. Crystallization from heptane resulted in colorless needles, m.p. 210°.

Anal. Calcd. for $C_{17}H_{14}F_3NO$: C, 66.9; H, 4.59. Found: C, 67.1; H, 4.72.

7-Ethyl-2-benzalaminofluorene.—The reaction of 0.42 g. of 7-ethyl-2-aminofluorene in 4 ml. of hot alcohol with 0.23 ml. of benzaldehyde gave the Schiff base on cooling. Crystallization from heptane gave a 93% yield of long yellow needles, m.p. 141–142°.

Anal. Calcd. for $C_{22}H_{19}N$: C, 88.9; H, 6.40; N, 4.71. Found: C, 89.1; H, 6.46; N, 4.68.

7-Ethyl-2-cinnamalaminofluorene.—This derivative was formed by the reaction between 7-ethyl-2-aminofluorene and cinnamaldehyde in alcohol. Crystallization from alcohol gave gleaming yellow crystals in 95% yield, m.p. 172–173°.

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Anal. Calcd. for $C_{24}H_{21}N$: C, 89.2; H, 6.50. Found: C, 89.0; H, 6.40.

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Alfalfa Saponin[1]

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Investigation of the water-soluble fraction of alfalfa (*Medicago sativa*), undertaken to test the hypotheses that it contains saponin capable of inhibiting growth of chicks[2] and contributing to ruminant bloat[3,4] confirmed earlier reports of the presence of at least two saponins.[5-8] Mixed saponins were recovered from dried alfalfa and have since been shown in a coöperative study by Heywang[9] to be inhibitory to the growth of chicks. In other recent coöperative experiments[10] the feeding of alfalfa saponin to ruminants caused typical symptoms of bloat.

Recovery of the mixed saponins from the plant material was effected through formation of their water-insoluble cholesterides. Because the cholesterides are split by alcohols,[7,8] it was necessary to form them by heating aqueous plant extract solutions containing an excess of cholesterol in suspension.

Partial resolution of the mixed saponins gave two fractions which differed from each other in optical rotation and mobility on paper and which by acid hydrolysis were also found to differ significantly from one another in both their sugar and aglycone components.

One of the sapogenins, its diacetate and diacetate dimethyl ester were prepared in crystalline form. Its dimethyl ester and monobromolactone were obtained as non-crystalline products. Properties of the sapogenin and its derivatives indicate that it is a monounsaturated dihydroxy dicarboxylic acid having the molecular formula $C_{30}H_{46}O_6$. The specific rotation of the sapogenin, $+111°$, and of its diacetate, $+87°$, suggests a triterpenoid, since the steroid-sapogenin side chain usually confers pronounced levorotation.[11]

The acidic character of the sapogenin and the fact that it contains 30 carbon atoms further support the idea that it belongs to the triterpenoid rather than the steroid class.[12]

A search of the literature disclosed no description of a sapogenin coinciding in all respects with that of the present substance. Castanogenin, a dihydroxy dicarboxylic acid sapogenin obtained by Simes[13] from the wood of *Castanospermum australe* apparently has the same molecular formula, $C_{30}H_{46}O_6$, as the sapogenin derived from alfalfa. However, melting points and specific rotations reported for the diacetate and the diacetate dimethyl ester differ from those of the corresponding substances prepared from alfalfa.

Experimental

Recovery of Saponin.—Dehydrated alfalfa meal (91 kg.) was extracted with 3 portions of hot water to yield 1,163 liters of solution, which was concentrated in a rising-film evaporator to yield 73 kg. of sirupy liquid. Ethanol (95%) was added to the concentrate to form an 80% alcohol mixture. The resulting precipitate was drained, suspended in 26.7 liters of water, and reprecipitated by adding 142.5 liters of 95% ethanol. This precipitate was drained and discarded. The combined alcoholic mother liquors were evaporated *in vacuo* to produce 26.4 kg. of aqueous concentrate. The concentrate was washed twice by mixing with $^1/_9$ its volume of chloroform and separating the chloroform solutions in a continuous centrifuge, leaving 24.3 kg. of washed concentrate.

The chloroform washed concentrate, in 3-kg. portions, was boiled with 720-g. portions of cholesterol and then mixed with filter aid and suction filtered. The filter cakes were washed with warm water until no more color was removed, then dried at 40°. Dried cake from each 3-kg. portion was leached with about 3 liters of anhydrous pyridine. Four volumes of anhydrous ether was added to the pyridine solution. The precipitated crude saponin was collected on a filter with light suction and washed with ether to remove pyridine and cholesterol, and then the product was dried to an amorphous white powder; yield 431 g.

Purification of Crude Saponin.—A 25-g. portion of the crude material was leached with 7 successive portions of boiling 95% ethanol. An undissolved dark brown residue that remained was discarded. Each solution was decanted and filtered hot. The first leach solution was found to contain more impurities than the others and was set aside. The remaining solutions were combined and evaporated to a volume of 1,250 ml. Thirty ml. of water was added to the hot mixture to redissolve the portion of material that came out of solution by concentration. Saponin was precipitated from the concentrate by adding 2 volumes of ether, filtered off with light suction, washed with ether and vacuum dried at 50°; yield 7.2 g.

Anal: sulfated ash, 0.04%; N, 0.09%; $[\alpha]^{26}D$ $-13.2°$ (*c* 1.0, water, *l* 4).

The product was moderately soluble in water and dilute ethanol but almost insoluble in absolute ethanol. Like the crude, it was strongly sternutatory and gave stable foams in water. Its 1% solution was faintly straw colored. The purified material hemolyzed defibrinated rat blood diluted with normal saline solution at slightly faster rates than did equal concentrations of a commercial saponin (J. T. Baker Chemical Co. lot No. 61543). Mosquito fish (*Gambuzia affinis*) placed in a solution of 1 g. of the product per liter died within 15 minutes, but a solution of $^1/_{10}$ of that concentration produced no effect on fish within a 8-hour test period.

Fractionation of Saponin.—Purified saponin (10.8 g.) was dissolved in 60 ml. of warm water. One liter of boiling 95% ethanol and 1.12 liters of boiling absolute ethanol were added to the solution. The precipitate, formed during addition of the absolute ethanol (fraction A), was filtered from the hot mixture, washed with hot 95% ethanol and dried; yield 3.3 g. The filtrate from fraction A was allowed to cool and stand overnight at room temperature. During this time a further quantity of precipitate formed. This was filtered off and 3 volumes of ether was added to the remaining solution. The resulting precipitate (fraction B), was recovered on a suction filter, washed with ether and dried; yield 4.55 g.

When tests showed that both fractions contained appreciable amounts of ash, 500-mg. portions of each were dissolved in water and demineralized by passage through columns of cation-exchange resin (2 g. each of Analytical Grade Amberlite IR-120 (H) and Duolite A-4 (OH)). The

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