Anal. Calcd. for $C_{18}H_{40}O_5NP$ (381.0): C, 56.69; H, 10.58; N, 3.67; P, 8.13. Found: C, 56.63; H, 10.48; N, 3.58; P, 7.61.

The alkali-purified material, 97.4 mg., was oxidized with periodic acid as previously described.[10] Consumption of periodate indicated 71% completion of the reaction; yield of 2,4-dinitrophenylhydrazone of palmitaldehyde 44.0 mg., m.p. 106–107°.

Acknowledgment. The author wishes to acknowledge the assistance of Mr. Wilson Woodbeck in the preparation of the beef spinal cord sphingolipides, Mrs. Florence Brand in the nitrogen and ultraviolet determinations, Mrs. Sonia Braun in the phosphorus analyses, and Mr. James Clark and Mrs. Mary Fusillo in preparing the sphingosine sulfate employed in this investigation.

New York, N. Y.

[Contribution from the Research Department of the R. J. Reynolds Tobacco Co.]

The Composition of Cigarette Smoke. V. Solanesenes

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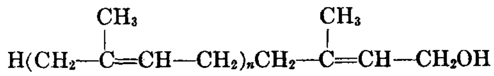
Received June 6, 1960

Similar series of solanesenes were obtained (a) from the cigarette smoke of a cased commercial blend of tobaccos, (b) by the dehydration of solanesol (I, $n = 8$), and (c) by the pyrolysis of solanesyl acetate (II, R = CH_3). Evidence is presented to indicate that a major portion of these solanesenes consists of 3-methylene-7,11,15,19,23,27,31,35-octamethyl-1,6,10,14,18,22,26,30,34-hexatriacontanonaene (III) and 3,7,11,15,19,23,27,31,35-nonamethyl-1,3,6,10,14,18,22,26,30,34-hexatriacontadecaene (IV).

In a continuation of our study of the condensable portion of cigarette smoke from a cased commercial blend of tobaccos,[1–4] a hydrocarbon fraction was isolated by chromatographic separation of a base-free hexane soluble fraction of cigarette smoke. Further chromatography yielded various phytadienes,[3] a solution of isomeric squalenes similar to that described by Kosak, *et al.*[5] and Van Duuren, *et al.*[6] in cigarette smoke plus several other fractions containing highly unsaturated aliphatic hydrocarbons. One of these latter fractions had an infrared absorption spectrum not too dissimilar from that reported for squalene regenerated from squalene hexahydrochloride using pyridine.[7] The major dissimilarity was in the presence of absorption at 6.25 μ, indicative of conjugated double bonds. Absorption at 6.01 μ and 11.92 μ (indicative of a trialkylethylene[8–11]), 6.07 μ (indicative of a terminal methylene group[8]), 7.23 μ (indicative of methyl groups[12]), 10.12 μ, 10.30 μ, 11.00 μ, 11.22 μ (indicative of the configuration $RR'C{=}CH_2$[9–11]) and 12.50 μ was noted for this fraction. This hydrocarbon fraction was sufficiently remote from the phytadiene fractions described elsewhere[3] in the chromatographic scheme to preclude contamination with these hydrocarbons as an explanation for the absorption at 6.25 μ. The elemental analysis of this material was in agreement with the empirical formula C_5H_8.

Hydrogenation of this fraction using platinum oxide as catalyst indicated ten double bonds for a molecular weight of 613 and yielded a saturated hydrocarbon whose elemental analysis, refractive index, and infrared absorption were identical with those of solanesane prepared by the catalytic hydrogenation of solanesol (I, $n = 8$).[13] This alcohol



$$H(CH_2{-}\overset{\displaystyle CH_3}{\overset{|}{C}}{=}CH{-}CH_2)_nCH_2{-}\overset{\displaystyle CH_3}{\overset{|}{C}}{=}CH{-}CH_2OH$$

I

has been reported as a constituent of flue-cured tobacco.[13] The structure originally postulated[13] for solanesol had $n = 9$ but this was recently re-

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[1] A. Rodgman and L. C. Cook, *Tobacco Science*, **3**, 86 (1959).

[2] A. Rodgman, L. C. Cook, and P. H. Latimer, *Tobacco Science*, **3**, 125 (1959).

[3] A. Rodgman, *J. Org. Chem.*, **24**, 1916 (1959).

[4] A. Rodgman and L. C. Cook, *Tobacco Science*, **4**, 7 (1960).

[5] (a) A. I. Kosak and J. S. Swinehart, *Chem. & Ind. (London)*, 1007 (1958). (b) A. I. Kosak and J. S. Swinehart, *J. Org. Chem.*, **25**, 222 (1960).

[6] B. L. Van Duuren and F. H. Schmitt, *Chem. & Ind. (London)*, 1006 (1958). These authors noted that the isolation of squalene from tobacco leaf had not been reported. Recently, Dr. J. D. Fredrickson of these laboratories isolated from burley tobacco a hydrocarbon whose infrared absorption was identical with that of authentic squalene.

[7] W. G. Dauben, H. L. Bradlow, N. K. Freeman, D. Kritchevsky, and M. Kirk, *J. Am. Chem. Soc.*, **74**, 4321 (1952).

[8] D. Barnard, L. Bateman, A. J. Harding, H. P. Koch, N. Sheppard, and G. B. B. M. Sutherland, *J. Chem. Soc.*, 915 (1950).

[9] R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 120 (1947).

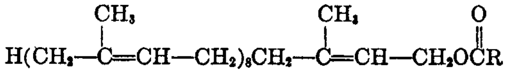
[10] N. Sheppard and G. B. B. M. Sutherland, *Proc. Royal Soc. (London)*, **A196**, 195 (1949).

[11] H. W. Thompson and D. H. Whiffen, *J. Chem. Soc.*, 1412 (1948).

[12] R. S. Rasmussen, *J. Chem. Phys.*, **16**, 712 (1948).

[13] R. L. Rowland, P. H. Latimer, and J. A. Giles, *J. Am. Chem. Soc.*, **78**, 4680 (1956). Reprinted in *Tobacco Science*, **1**, 86 (1957).

vised[14–17] to $n = 8$. Various derivatives of solanesol (I, $n = 8$) have also been identified in flue-cured tobacco, e.g., various solanesyl esters (II) of long chained fatty acids,[18] solanesyl acetate (II, R = CH_3)[17] and solanachromene.[19]



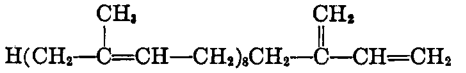
$$\mathrm{H(CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2)_8CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2O\overset{O}{\overset{\|}{C}}R}$$

II

Confirmation of the relationship of the structure of the compound or compounds constituting the hydrocarbon fraction from cigarette smoke to that of solanesol was provided by the results of experiments involving the dehydration of solanesol (I, $n = 8$) and the pyrolysis of solanesyl acetate (II, R = CH_3). Dehydration of solanesol in benzene at 70° using potassium hydrogen sulfate yielded an unsaturated hydrocarbon fraction whose infrared absorption was almost identical with that of the smoke fraction. Due to the relationship of this hydrocarbon fraction to solanesol, we have called it solanesene, a name previously suggested by Rowland.[19]

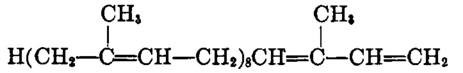
Pyrolysis of solanesyl acetate (II, R = CH_3) at 400° in a nitrogen atmosphere using a flow-type pyrolysis system yielded a hydrocarbon fraction whose infrared absorption was identical with that of the solanesene fraction obtained either from cigarette smoke or from solanesol by dehydration. The pyrolysis of an ester to yield a carboxylic acid and an ethylene is well known. Similarly, esters of allylic alcohols have been shown to undergo 1,4-elimination of a carboxylic acid on pyrolysis to yield the acid plus a conjugated diene.[20,21]

Elucidation of the structure of the solanesene(s) was accomplished as follows: Sodium-alcohol reduction of the cigarette smoke fraction produced a marked decrease in the absorption at 6.25 and 11.22 μ in relation to the other absorption bands. This was interpreted as being due to reduction of conjugated double bonds involving a terminal methylene group to an isolated double bond in an appreciable percentage of the material. Attempts to separate the reduced material from the starting material by chromatography were unsuccessful. On the basis of the infrared and reduction studies, we postulated that the hydrocarbon fraction isolated from smoke contained several solanesenes, of which the compounds 3-methylene-7,11,15,19,23,27,31,35-octamethyl-1,6,10,14,18,22,26,30,34-hexatriacontanonaene (III) and 3,7,11,15,19,23,27,31,35 - nonamethyl - 1,3,6,10,14,18,22,26,30,34-hexatriacontadecaene (IV) constituted the major percentage.



$$\mathrm{H(CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2)_8CH_2{-}\overset{CH_2}{\overset{\|}{C}}{-}CH{=}CH_2}$$

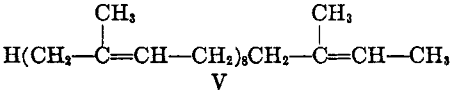
III



$$\mathrm{H(CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2)_8CH{=}\overset{CH_3}{\overset{|}{C}}{-}CH{=}CH_2}$$

IV

The hydrocarbon obtained by sodium-alcohol reduction of the material considered to be mostly III and IV was assigned structure V and called dihydrosolanesene (3,7,11,15,19,23,27,31,35-nonamethyl - 2,6,10,14,18,22,26,30,34 - hexatriacontanonaene).



$$\mathrm{H(CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2)_8CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_3}$$

V

Evidence supporting the structures advanced for the components (III plus IV) of the solanesene fraction was provided by ozonization studies. Ozonization of the solanesenes obtained either from cigarette smoke or by dehydration of solanesol (I, $n = 8$), followed by reductive cleavage of the ozonide, yielded acetone, levulinaldehyde, methylglyoxal, and formaldehyde, identified as 2,4-dinitrophenylhydrazones. Similar studies of the sodium-alcohol reduction product, presumed to be V (and still contaminated with some III and IV), yielded acetone, levulinaldehyde, a trace of formaldehyde, and acetaldehyde, identified as 2,4-dinitrophenylhydrazones. These data are consistent with the structures advanced as III and IV for the solanesene fraction and as V for dihydrosolanesene. Table I compares the results obtained by ozonization of the solanesene fraction (III plus IV) from cigarette smoke and from solanesol and by ozonization of dihydrosolanesene (V).

Since the Diels-Alder reaction had been used with some success in the identification of neophytadiene from flue-cured tobacco[22] and the identification of neophytadiene and other phytadienes from cigarette smoke,[3] it was employed in the study of the solanesene fraction obtained in the present investigation. Treatment of the material isolated from cigarette smoke with 1,4-naphthoquinone (VI) followed by air oxidation gave a very low yield of a monosubstituted anthraquinone presumed to be VII. This material was a pale yellow oil whose infrared absorption was different from those obtained with the air oxidized phytadiene-1,4-

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[14] R. E. Erickson, C. H. Shunk, N. R. Trenner, B. H. Arison, and K. Folkers, *J. Am. Chem. Soc.*, **81**, 4999 (1959).

[15] C. H. Shunk, R. E. Erickson, E. L. Wong, and K. Folkers, *J. Am. Chem. Soc.*, **81**, 5000 (1959).

[16] M. Kofler, A. Langemann, R. Rüegg, U. Gloor, U. Schweiter, J. Würsch, O. Wiss, and O. Isler, *Helv. Chim. Acta*, **42**, 2252 (1959).

[17] R. L. Rowland and J. A. Giles, *Tobacco Science*, **4**, 29 (1960).

[18] R. L. Rowland and P. H. Latimer, *Tobacco Science*, **3**, 1 (1959).

[19] R. L. Rowland, *J. Am. Chem. Soc.*, **80**, 6130 (1958).

[20] W. J. Bailey and R. Barclay, *J. Org. Chem.*, **21**, 328 (1956).

[21] F. L. Greenwood, *J. Org. Chem.*, **24**, 1735 (1959).

[22] R. L. Rowland, *J. Am. Chem. Soc.*, **79**, 5007 (1957).

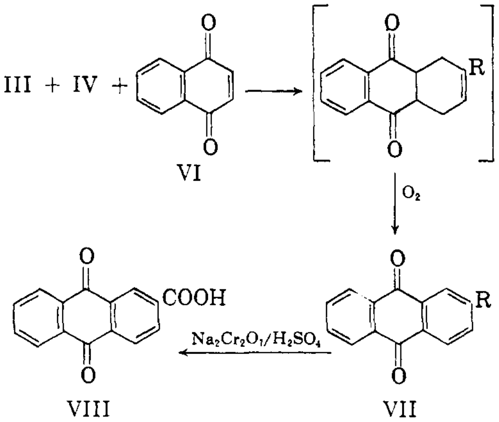
TABLE I

OZONIZATION STUDIES

2,4-Dinitrophenyl-hydrazone of	Quantities of 2,4-Dinitrophenylhydrazones Obtained by Reductive Cleavage of the Ozonide of: Solanesenes from Cigarette Smoke (0.450 g.) Calcd. If III g.	If IV g.	Found g.	Solanesenes from Solanesol (0.576 g.) Calcd. If III g.	If IV g.	Found g.	Dihydrosolanesene (V) (0.226 g.) Calcd. g.	Found g.
Formaldehyde	0.310	0.155	0.086	0.396	0.198	0.048	0	Trace
Acetaldehyde	0	0	0	0	0	0	0.083	0.022[f]
Acetone	0.175	0.175	0.145[a]	0.224	0.224	0.207[d]	0.088	+
Methylglyoxal	0	0.318	0.033	0	0.407	+	0	[c]
Levulinaldehyde	2.490	2.490	2.10[b]	3.030	3.030	2.82[e]	1.350	1.23[g]
Malonaldehyde	0	0.318	[c]	0	0.407	[c]	0	[c]
α-Ketoglutaraldehyde	0.480	0	[c]	0.615	0	[c]	0	[c]

[a] 83% yield. [b] 84% yield. [c] Not determined. [d] 92% yield. [e] 93% yield. [f] 26% yield. [g] 91% yield.

naphthoquinone adducts.[3,22] Oxidation of VII with sodium dichromate in sulfuric acid yielded anthraquinone-2-carboxylic acid (VIII). The low yield obtained in the initial steps of this reaction sequence is probably due to the relative inertness of these high molecular weight hydrocarbons in the Diels-Alder reaction.



R = H[CH_2—C(CH_3)=CH—CH_2]$_8$$CH_2$—

Since anthraquinone-2-carboxylic acid (VIII) was the only acid found, it is presumed that IV was too inert to react. This finding is in agreement with results obtained in the Diels-Alder reaction involving 1,4-naphthoquinone and various phytadienes.[3,22]

Demonstration that small traces of neophytadiene (the most difficult phytadiene to elute from alumina[3,22]) did not contribute to the formation of a monosubstituted anthraquinone similar to VII which would be oxidizable to VIII was provided by the results of the following study: Since solanesol is relatively polar compared with neophytadiene, separation of these two compounds is accomplished with great ease by chromatography using alumina. The solanesene fraction obtained by dehydration of pure solanesol on treatment with 1,4-naphthoquinone in the Diels-Alder reaction, followed by air oxidation and sodium dichromate–sulfuric acid oxidation, gave a low yield of anthraquinone-2-carboxylic acid as in the case of the smoke solanesenes.

The squalenes isolated from cigarette smoke in our study comprised 0.00034% of the total weight of the tobacco smoked [0.00039% (moisture free basis), or 2.4 mg. per 1000 cigarettes]. This value is approximately three times that reported by Kosak, *et al.*[5a] which is reasonable since our smoking rate was a two-second puff per twenty seconds in comparison with a two-second puff per minute used by Kosak, *et al.*[5,23]

The solanesenes isolated from cigarette smoke comprised 0.0085% of the total weight of the tobacco smoked [0.0094% (moisture free basis), or 60 mg. per 1000 cigarettes]. Our studies suggest that the precursors in the tobacco of the solanesenes in cigarette smoke may be solanesol and the solanesyl esters. If this be the case, we then have an explanation for at least a portion of the difference between the quantities of solanesol[13] and solanesyl esters[17,18] in tobacco and the quantities of these substances in cigarette smoke.[1,2,24]

EXPERIMENTAL[25]

Isolation of squalenes from cigarette smoke. The smoking apparatus and procedure, and the chemical fractionation and preliminary chromatography of the smoke condensate have been described previously.[1-3]

The hydrocarbon fractions, designated previously[3] as *Fractions 6 and 7* (total wt., 17.28 g.), which were obtained

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(23) A. I. Kosak, J. S. Swinehart, and D. Taber, *J. Natl. Cancer Inst.*, 17, 375 (1956).

(24) J. D. Mold and J. S. Booth, *Tobacco Science*, 1, 38 (1957).

(25) All melting points were determined on a Fisher-Johns melting point apparatus. Elemental analyses were performed by the Huffman Microanalytical Laboratories, Wheatridge, Colo. Infrared absorption spectra were determined using a Perkin-Elmer Model 21 Spectrophotometer. Ultraviolet absorption spectra were determined using a Beckman DK2 Ratio Recording Spectrophotometer. The alumina employed was Aluminum Oxide Merck (Merck Cat. No. 71707); the silicic acid was obtained from Mallinckrodt Chemical Works (Cat. No. 2844).

from the isolation of phytadienes from 52,000 cigarettes, were chromatographed on alumina [45 mm. (diam.) × 200 mm.] using hexane as eluent. The initial 100-ml. fraction contained neophytadiene, 1.45 g. The next two fractions were solutions of neophytadiene and other unsaturated hydrocarbons, possibly isomeric squalenes. The fourth fraction, 0.156 g., contained a colorless oil whose infrared spectrum was identical with that of the squalenes regenerated from squalene hexahydrochloride using pyridine.[7] Subsequent fractions from this chromatogram will be described under solanesenes.

Rechromatography of the squalene fraction (0.156 g.) on alumina yielded 125 mg. of a colorless liquid, n_D^{27} 1.4923. This material had an infrared spectrum[7] identical with that of a sample of isomeric squalenes regenerated from squalene hexahydrochloride using pyridine according to the method of Heilbron, *et al.*,[26] and quite similar to that of the isomeric squalenes regenerated from squalene hexahydrochloride using sodium ethoxide.

Anal. Calcd. for $C_{30}H_{50}$: C, 87.75; H, 12.27. Found: C, 87.69; H, 12.28.

The squalenes isolated from cigarette smoke were converted[26] to squalene hexahydrochloride, m.p. 113–116°. This material had an infrared spectrum identical with that of squalene hexahydrochloride prepared from (a) pure squalene, (b) the squalenes regenerated from squalene hexahydrochloride using pyridine,[7,26] and (c) the squalenes regenerated from squalene hexahydrochloride using sodium ethoxide.

Anal. Calcd. for $C_{30}H_{56}Cl_6$: C, 57.80; H, 8.08; Cl, 34.1. Found: C, 57.51, 57.47; H, 8.98, 8.84; Cl, 33.7.

Isolation of solanesenes. A. From cigarette smoke. The fractions succeeding the squalenes in the chromatogram described above contained other unsaturated terpenoid hydrocarbons. Several fractions; namely, the sixth, seventh, and eighth, exhibited an infrared spectrum quite similar to that of the squalenes. The main difference was the absorption at 6.25 μ, indicative of conjugation. Other infrared absorptions were noted at 6.01, 6.07, 7.23, 10.12, 10.30, 11.00, 11.22, and 12.50 μ. The ultraviolet absorption determined in cyclohexane showed a maximum at 210 mμ log ε 4.25 and a shoulder at 230–236 mμ log ε 3.51. The total weight in these fractions was 4.55 g. Rechromatography on alumina permitted separation of the solanesenes (3.10 g.) as a pale yellow liquid, n_D^{27} 1.5143.

Anal. Calcd. for $C_{45}H_{72}$: C, 88.16; H, 11.84. Found: C, 88.31; H, 12.02.

B. From solanesol. A mixture of solanesol (2.14 g.), potassium hydrogen sulfate (4.28 g.), and benzene (15 ml.) was maintained at a temperature of 70–75° for 4 hr. The reaction mixture was cooled, diluted with hexane, filtered, and the filtrate concentrated to dryness to yield 2.11 g. of a pale yellow oil. Chromatography on alumina yielded two main fractions; namely, solanesenes (1.05 g.) and solanesol (0.73 g.). The infrared and ultraviolet absorption spectra of this sample of solanesenes were identical with those of the material isolated from cigarette smoke.

C. From solanesyl acetate. Solanesol (3.5 g.), isolated from flue-cured tobacco by the method of Rowland, *et al.*,[13] was converted to the acetate. The acetate was purified by chromatography on alumina.

The solanesyl acetate (1.5 g.), preheated to 60°, was permitted to drip through a vertical quartz pyrolysis tube onto quartz chips maintained at a temperature of 400 ± 3°. The pyrolysis tube was continuously swept with a stream of nitrogen. The pyrolysis product was separated by chromatography using alumina into two main fractions. The first fraction (0.3 g.), eluted by hexane, was a pale yellow oil with infrared absorption identical with that of the solanesene fraction isolated from cigarette smoke and with that prepared from solanesol in B above. The second fraction was mostly unchanged solanesyl acetate.

Reduction of solanesenes. A. To solanesane. The solanesenes (0.161 g.) isolated from cigarette smoke were quantitatively hydrogenated in ethanol using platinum oxide as catalyst. Ten double bonds for a molecular weight of 613 were indicated. Removal of the catalyst by filtration, followed by concentration of the filtrate yielded 0.160 g. of a colorless, viscous oil, n_D^{27} 1.4578. Chromatography using alumina gave 0.156 g. of a colorless viscous oil with the same refractive index as the crude product. Rowland, *et al.*,[13] reported solanesane with n_D^{26} 1.4582. The infrared absorption indicated a highly methylated, saturated hydrocarbon.

Anal. Calcd. for $C_{45}H_{92}$: C, 85.38; H, 14.62. Found: C, 85.43; H, 14.57.

B. To dihydrosolanesene. Solanesenes (1.23 g.) isolated from cigarette smoke were refluxed in 30 ml. of ethyl alcohol and 0.5 g. of metallic sodium was added at the rate of 0.1 g. per 3 min. After completion of the addition of the sodium, the reaction mixture was refluxed for 0.5 hr. The cooled reaction mixture was diluted with water, acidified with 2*N* hydrochloric acid and extracted with hexane (150 ml.). Concentration of the hexane extract yielded 1.05 g. of a pale yellow oil. The infrared spectrum of this material showed very little absorption at 6.25 μ and the absorption at 11.22 μ had decreased appreciably relative to the other absorption bands. The decrease in the absorption at 6.25 μ was interpreted as an indication of the reduction of the conjugated system to an isolated one.

Reduction of the ozonide of the solanesenes. A. From cigarette smoke. The ozonization of 0.450 g. of solanesenes isolated from cigarette smoke was accomplished in 100 ml. of ethyl acetate cooled in an ice-salt bath. The ozonide was reduced using 0.5 g. of zinc dust and 100 ml. of water.[27] The reduction products were treated with 550 ml. of 2*N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The 2,4-dinitrophenylhydrazones were recovered by filtration, washed with 2*N* hydrochloric acid and water, and then dried.

Extraction with hexane in a Soxhlet extractor yielded 0.34 g. of hexane-soluble and 2.47 g. of hexane-insoluble 2,4-dinitrophenylhydrazones.

Chromatography of the hexane-soluble 2,4-dinitrophenylhydrazones using silicic acid yielded acetone 2,4-dinitrophenylhydrazone, 0.145 g., m.p. 123–124°, and formaldehyde 2,4-dinitrophenylhydrazone, 0.086 g., m.p. 163.0–164.5°. Crystallization of the acetone derivative from ethanol raised the melting point to 124.0–125.0°. No depression of melting point was noted with an authentic sample. The infrared spectra of the authentic sample and that from the ozonide were identical.

Crystallization of the formaldehyde 2,4-dinitrophenylhydrazone from ethanol raised the melting point to 166°. No depression of melting point was noted with an authentic sample. The infrared spectra of the authentic sample and that from the ozonide were identical.

Crystallization of the hexane-insoluble 2,4-dinitrophenylhydrazones from acetonitrile yielded 2.10 g. of levulinaldehyde bis-2,4-dinitrophenylhydrazone, m.p. 234–236°. Mixture melting point with an authentic sample gave no depression. The infrared spectra of the authentic and isolated samples were identical.

Chromatography of the filtrate from the above crystallization yielded impure levulinaldehyde bis-2,4-dinitrophenylhydrazone plus 0.033 g. of methylglyoxal bis-2,4-dinitrophenylhydrazone, m.p. 297–300° dec. Crystallization of the latter fraction from nitromethane yielded material melting at 299–301° dec. A mixture melting point study with an authentic sample of methylglyoxal bis-2,4-dinitrophenylhydrazone gave no depression. The infrared spectra of the authentic and isolated samples were identical.

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(26) I. M. Heilbron, E. D. Kamm, and W. M. Owens, *J. Chem. Soc.*, 1630 (1926).

(27) G. A. Howard and A. R. Tatchall, *J. Chem. Soc.*, 2400 (1954).

Anal. Calcd. for $C_{15}H_{12}N_8O_8$: N, 25.86. Found: N, 25.90. Table I summarizes the results of this study.

B. From solanesol. Following ozonization of 0.576 g. of the solanesenes prepared from solanesol, the procedure described in A was repeated. The 2,4-dinitrophenylhydrazones of the following carbonyl compounds were isolated: acetone, 0.207 g., m.p. 123.5–124.5°; formaldehyde, 0.048 g., m.p. 166°; levulinaldehyde, 2.82 g., m.p. 237–238°; and methylglyoxal, trace. Infrared absorption studies confirmed the identity of these compounds. Table I summarizes these findings.

Reduction of the ozonide of dihydrosolanesene. Following ozonization of 0.226 g. of dihydrosolanesene, the procedure described in A was repeated. The 2,4-dinitrophenylhydrazones of the following carbonyl compounds were isolated: acetone, acetaldehyde, 0.022 g., m.p. 166–167°; levulinaldehyde, 1.23 g., m.p. 235–237°; and formaldehyde, trace. The infrared spectra of these compounds were identical with those of authentic samples. Table I summarizes the results obtained.

Diels-Alder reaction of 1,4-naphthoquinone with the solanesenes. A. From cigarette smoke. A solution of solanesenes (1.00 g.) and 1,4-naphthoquinone (1.00 g.) in 1:1 ethanol-benzene was refluxed for 5 hr. A solution of 300 mg. of potassium hydroxide in 30 ml. of ethanol was added and the resulting mixture was aerated for 6.5 hr. Concentration to dryness, extraction with hexane (100 ml.), followed by concentration of the hexane extract yielded 0.69 g. of a pale yellow oil.

Chromatography using silicic acid yielded 0.59 g. of unchanged solanesenes and 0.049 g. of a substituted anthraquinone whose infrared spectrum was substantially different from those of the anthraquinones derived from neophytadiene or other phytadienes.[3,22] Oxidation of the solanesene-derived anthraquinone was accomplished by solution in 1.5 ml. of sulfuric acid, addition of 0.5 g. of sodium dichromate in 1.5 ml. of water, and heating at 90° for 1 hr. Dilution of the reaction mixture with water followed by extraction with benzene yielded 7 mg. of a carboxylic acid. Chromatography of this material on silicic acid yielded 6 mg. of anthraquinone-2-carboxylic acid, m.p. 285–287°. Crystallization from 4:1 ethanol-water raised the melting point to 288–290°. Melting points of 291–292°,[28,29] 292–293°,[22] and 295–296°[3] have been reported. A mixture melting point with an authentic sample gave no depression. The infrared spectra of this material and that of an authentic sample were identical.

B. From solanesol. Repetition of the reaction sequence described in A using the solanesenes (1.0 g.) prepared from solanesol yielded 11 mg. of anthraquinone-2-carboxylic acid, m.p. 289–291°. A mixture melting point with an authentic sample gave no depression.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S.A.]

Steroids. CLIV. Alkyl Steroidal D-Ring Lactones[1,2]

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Condensation of 3β,13α-dihydroxy-13,17-secoandrostan-17-oic acid lactone, 3-keto-13α-hydroxy-13,17-oic acid and its lactone with ethyl formate and methoxide afforded respectively the 16-hydroxymethylene, 2-hydroxymethylene, and 2,16-bishydroxymethylene lactones which were catalytically hydrogenated to the 16β-methyl, 2α-methyl, and 2α,16β-dimethyl derivatives. Oxidation of the 16β-methyl-3β,13α-dihydroxy-13,17-secoandrostan-17-oic acid lactone yielded the 3-keto derivative. Conversion of the methyl substituted 3-keto steroidal lactones to Δ^1 and $\Delta^{1,4}$-3-keto lactones is described.

Since Westerfeld's original observation that oxidation of estrone with alkaline hydrogen peroxide afforded a D-ring lactone[3] the peracid oxidation of other 17-keto steroids has been reported[4–8] and the resulting lactones unequivocably established as 13,17-seco steroidal lactones (I) rather than 16,17-seco lactones (II).[8] Biological oxidation of various steroidal substrates has been reported also to produce related D-Ring lactones.[9–11]

In this paper we report the synthesis of a number of C-2 and C-16 methyl-substituted derivatives of testololactone. Our interest in these derivatives stems in part from the reported[12] favorable activity of 2α-methylandrostane-17β-ol-3-one[13] and of 1-

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