both fatty acids; dihydronicotinamide-adenine dinucleotide dehydrogenase was inhibited by 85 % and 92 % by 1 mM-stearate and 1 mM-oleate respectively.

9. The results are discussed in relation to the general problems of isolating 'intact' plant mitochondria and the action of fatty acids in inhibiting mitochondrial functions.

The authors are indebted to Dr F. D. Collins for valuable discussions. L.D. held a Sir John and Lady Higgins Research Scholarship from the University of Melbourne during this work. The Pye Chromatograph was used by courtesy of F. V. Harold and A. S. Morieson, of Carlton and United Breweries.

REFERENCES

- Ahmed, K. & Scholefield, P. G. (1961). *Biochem. J.* **81**, 37.
 Allen, R. J. L. (1940). *Biochem. J.* **34**, 858.
 Baker, P. R., Northcote, D. H. & Peters, R. (1962). *Nature, Lond.*, **195**, 661.
 Bevers, H. (1961). *Nature, Lond.*, **191**, 433.
 Bellamy, D. & Bartley, W. (1960). *Biochem. J.* **76**, 78.
 Bendall, D. S. & de Duve, C. (1960). *Biochem. J.* **74**, 444.
 Bonner, W. D. & Voss, D. O. (1961). *Nature, Lond.*, **191**, 682.
 Boyer, P. D., Ballou, G. A. & Luck, J. M. (1947). *J. biol. Chem.* **167**, 407.
 Cleland, K. W. & Slater, E. C. (1953). *Biochem. J.* **53**, 547.
 Dalgarno, L. & Birt, L. M. (1962a). *Biochem. J.* **83**, 195.
 Dalgarno, L. & Birt, L. M. (1962b). *Biochem. J.* **83**, 202.
 Dalgarno, L. & Birt, L. M. (1963). *Biochem. J.* **86**, 46.
 Dawson, R. M. C. (1962). In *Comparative Biochemistry*, vol. 3, p. 267. Ed. by Florkin, M. & Mason, H. S. New York and London: Academic Press.
 Dervichian, D. G. (1954). *Progr. Chem. Fats & Lipids*, **2**, 193.
 Emmelot, P. (1962). *Cancer Res.* **22**, 38.
 Emmelot, P. & Bos, C. J. (1961). *Brit. J. Cancer*, **15**, 373.
 Farquhar, J. W., Insull, W., Rosen, P., Stoffel, W. & Ahrens, E. H. (1959). *Nutr. Rev.* **17** (suppl.), 1.
 Gilmour, D. (1961). *The Biochemistry of Insects*, p. 100. New York and London: Academic Press.
 Goodman, D. S. (1958). *J. Amer. chem. Soc.* **80**, 3892.
 Grafflin, A. L. & Green, D. E. (1948). *J. biol. Chem.* **176**, 95.
 Green, D. E. & Fleischer, S. (1960). In *Metabolic Pathways*, vol. 1, p. 41. Ed. by Greenberg, D. M. New York and London: Academic Press.
 Hackett, D. P., Rice, B. & Schmid, C. (1960). *J. biol. Chem.* **235**, 2140.
 Helinski, D. R. & Cooper, C. (1960). *J. biol. Chem.* **235**, 3573.
 Hilditch, T. P. (1941). *The Chemical Constitution of Natural Fats*, p. 145. London: Chapman and Hall Ltd.
 Hulsmann, W. C., Elliott, W. B. & Slater, E. C. (1960). *Biochim. biophys. Acta*, **39**, 267.
 Kennedy, E. P. & Lehninger, A. L. (1949). *J. biol. Chem.* **179**, 957.
 Lardy, H. A. & Wellman, H. (1952). *J. biol. Chem.* **195**, 215.
 Lehninger, A. L. & Remmert, L. F. (1959). *J. biol. Chem.* **234**, 2459.
 Lewis, S. E. & Slater, E. C. (1954). *Biochem. J.* **58**, 207.
 Meyer, H., Preiss, B. & Bauer, Sh. (1960). *Biochem. J.* **76**, 27.
 Pierpoint, W. C. (1960). *Biochem. J.* **75**, 504.
 Polis, B. D. & Shmukler, H. W. (1957). *J. biol. Chem.* **227**, 419.
 Racker, E. (1950). *J. biol. Chem.* **184**, 313.
 Roodyn, D. B. & Suttie, J. W. (1961). *Biochim. biophys. Acta*, **52**, 376.
 Sacktor, B. (1955). *J. biophys. biochem. Cytol.* **1**, 29.
 Sacktor, B., O'Neill, J. J. & Cochran, D. G. (1958). *J. biol. Chem.* **233**, 1233.
 Singer, T. P. & Hofstee, B. H. J. (1948). *Arch. Biochem.* **18**, 229.
 Stern, I. & Shapiro, B. (1953). *J. clin. Path.* **6**, 158.
 Stoffel, W., Chu, F. & Ahrens, E. H. (1959). *Analyt. Chem.* **31**, 307.
 Stumpf, P. K. & Barber, G. A. (1956). *Plant Physiol.* **31**, 304.
 Stumpf, P. K. & Bradbeer, C. (1959). *Annu. Rev. Plant Physiol.* **10**, 197.
 Weinbach, E. C. (1959). *J. biol. Chem.* **234**, 1580.
 Wojtczak, L. & Lehninger, A. L. (1961). *Biochim. biophys. Acta*, **51**, 442.

Biochem. J. (1963) **87**, 596

Identification of the D-Glucosides of Stigmasterol, Sitosterol and Campesterol in Tobacco and Cigarette Smoke

By A. G. KALLIANOS, F. A. SHELBURNE, R. E. MEANS, R. K. STEVENS, R. E. LAX
 AND J. D. MOLD

Liggett and Myers Tobacco Co., Research Department, Durham, North Carolina, U.S.A.

(Received 16 October 1962)

Several sterols and sterol esters have been reported in tobacco and in cigarette smoke. Reports on the presence of stigmasterol, β -sitosterol and γ -sitosterol in tobacco and cigarette smoke have been cited in a review by Johnstone & Plimmer

(1959). The presence of these sterols in cigarette smoke has been confirmed by Kosak & Swinehart (1960). Kosak, Swinehart, Taber & Van Duuren (1957) and Grossman & Stedman (1958), in reporting the occurrence of free stigmasterol in

tobacco smoke and flue-cured tobacco respectively, proposed the possibility that this compound may have occurred as the glucoside. Campesterol has also been reported to occur in flue-cured tobacco by Dymicky & Stedman (1959a).

In addition to the free sterols, fatty acid esters of stigmaterol and β -sitosterol were reported to be present in cigarette smoke by Rodgman, Cook & Latimer (1959). The sterols were isolated as a mixture and the stigmaterol: β -sitosterol ratio was approximately 4:1.

Sterol glycosides have not been found in smoke; however, they have been isolated from tobacco. Dymicky & Stedman (1958) isolated β -sitosterol D-glucoside and a partially characterized steroid glucoside (Dymicky & Stedman, 1959b) from flue-cured tobacco leaves. A glucoside of γ -sitosterol was reported in Indian chewing tobacco by Khanolkar, Panse & Divekar (1955). Divekar, Ambaye, Panse & Khanolkar (1961) reported the isolation of four sterol glucosides from sun-dried Indian tobacco; no assignment of structures was made, however. Wright, Burton & Berry (1962) reported the presence of stigmaterol D-glucoside and presented mass-spectra data on sterols from the hydrolysate of a sterolin mixture from aged Burley tobacco indicating the probable presence of the glucosides of β -sitosterol and of campesterol.

The present paper describes the isolation and identification of the D-glucosides of stigmaterol, sitosterol and campesterol from tobacco and cigarette smoke. These compounds were isolated as a mixture from tobacco and cigarette smoke. The mixture had the same physical and chemical properties as those of the partially characterized steroid glucoside (Dymicky & Stedman, 1959b).

EXPERIMENTAL

Melting points are corrected.

Optical rotations were determined in a 1 dm. tube by the use of a Zeiss Circle Polarimeter reading to 0.01°. The precision of the measurements for $[\alpha]_D$ were estimated to be $\pm 1.2^\circ$.

Authentic sterols used as reference. The stigmaterol and β -sitosterol were obtained from Mann Research Laboratories Inc., New York, N.Y., U.S.A. The materials were recrystallized from 95% (v/v) ethanol to give stigmaterol, m.p. 168°, $[\alpha]_D^{26} - 49.0^\circ$, and β -sitosterol, m.p. 138°, $[\alpha]_D^{26} - 37.0^\circ$ [Fieser & Fieser (1959) reported: stigmaterol, m.p. 170°, $[\alpha]_D - 51^\circ$; β -sitosterol, m.p. 140°, $[\alpha]_D - 37.0^\circ$]. The campesterol was obtained from Dr Humphlett of Distillation Products Industries, Rochester, N.Y., U.S.A., and was further purified by several recrystallizations from hexane, m.p. 156°, $[\alpha]_D^{24} - 34.3^\circ$ [Fieser & Fieser (1959) reported: campesterol, m.p. 158°, $[\alpha]_D - 33^\circ$].

Isolation of sterol glucoside from cigarette smoke. Regular-length (70 mm.) cigarettes of a cased commercial blend of flue-cured, Burley, Maryland and Turkish tobaccos were

smoked mechanically on a constant-puff-volume cigarette-smoking machine. Puffs (35 ml.), each lasting 2 sec., were taken at 1 min. intervals from each cigarette. Eight puffs were required to smoke each cigarette to an average butt length of 30 mm. The smoke was condensed in a series of three all-glass traps cooled in liquid air. The condensed material was rinsed out of the traps with diethyl ether, water and hexane. A total average yield of 20.9 g. of condensed material, which was relatively non-volatile at 25°, was obtained from 1000 cigarettes. The solvents were combined and concentrated to remove the ether, and the resulting mixture was extracted with hexane. The hexane layers were filtered and utilized for other purposes. The precipitate and emulsion obtained from the hexane extraction of smoke from 100 000 cigarettes (231 g.) was dissolved in acetone and concentrated *in vacuo* at 50°. This concentrate was passed through a percolator packed with 454 g. of Analytical-Grade Celite (Johns-Manville Co.), followed by 1400 ml. of water and several litres of ether. The combined effluent from the percolation with water was extracted with ether. The ether extracts were combined with the ether rinse from the percolation and concentrated *in vacuo* to 750 ml., containing 152 g. of non-volatile solids. This solution was extracted with three 750 ml. portions of 0.01 N-hydrochloric acid and three 750 ml. portions of 0.05 N-hydrochloric acid. The pH of the final aqueous phase to contact the ether was 1.75. On concentrating the ether solutions and rinsing with acetone, an insoluble white material separated. The yield of crude crystalline product was 159.4 mg. (1.59 mg./1000 cigarettes), m.p. above 280° (decomp.). Purification was accomplished through several recrystallizations from ethanol to give white crystals, m.p. 288–291° (decomp.), $[\alpha]_D^{25} - 41.0^\circ$ (c 2.67 in pyridine). A positive Liebermann–Burchard reaction, with emerald-green coloration, was obtained (Found: C, 72.5, 72.5; H, 10.5, 10.1%). The infrared-absorption spectrum of the material indicated a glycoside. The infrared-absorption spectra of this and subsequent samples were determined, by using the KBr-pressed-disk technique, with a Perkin–Elmer model 21 infrared spectrometer equipped with NaCl optics.

Isolation of sterol glucoside from tobacco. An uncased commercial blend of Burley, Bright, Maryland and Turkish tobaccos (1825 g.) was extracted with light petroleum (b.p. 40–48°) and methanol in a percolator heated at 10° below the boiling point of the solvent. The light petroleum extract contained 87.04 g. of non-volatile solids (5.3% of the dry weight of tobacco) and the methanol extract contained 243.8 g. of non-volatile solids (14.9% of the dry weight of tobacco). The latter solution was diluted with water and extracted into ether. The ether extract was concentrated, water was added and several ether extractions were performed. The ether extract contained 41.6 g. of non-volatile solids (2.53% of the dry weight of tobacco). In the last step of this procedure, when water was added to the ether concentrate, a white flocculent precipitate separated at the interface. This was collected by filtration, dried, dissolved in methanol, decolorized with charcoal, filtered and allowed to cool. The white crystals that separated were recrystallized three more times from 95% (v/v) ethanol to give 0.12 g. (0.0073% of the dry weight of tobacco) of material, m.p. 293–294° (decomp.), $[\alpha]_D^{25} - 40.0^\circ$ (c 2.6 in pyridine). The material gave a positive Liebermann–Burchard reaction (Found: C, 73.0, 72.8; H, 10.5, 10.4%).

The infrared-absorption spectrum of this material was the same as that of the material isolated from cigarette smoke. The yields of crude sterol glycoside for two preparations in which waxy materials were washed out with light petroleum were respectively 0.043 and 0.045% of the dry weight of tobacco.

Hydrolysis of the sterol glycoside. The sterol glycoside was hydrolysed with acid (1 ml. of conc. sulphuric acid/100 ml. of ethanol) by the method of Swift (1952). The sterol moiety was extracted with ether and the solvent was removed *in vacuo*; a white precipitate was deposited. After three recrystallizations from 95% (v/v) ethanol the smoke sterol had m.p. 142°, $[\alpha]_D^{24} - 39.4^\circ$ (c 2.6 in chloroform). The tobacco sterol had m.p. 143–144°, $[\alpha]_D^{25} - 43.4^\circ$ (c 3.15 in chloroform) (Found: C, 83.6, 83.4; H, 11.9, 12.3%).

Identification of the sugar. The aqueous layer of the hydrolysate was adjusted to pH 6 with basic ion-exchange resin, Dowex 1 (200–400 mesh) (Dow Chemical Co.). After filtration, the solvent was removed by freeze-drying. A sample of the residue after the freeze-drying was spotted on Whatman no. 1 paper and analysed by paper electrophoresis (Briggs, Garner, Montgomery & Smith, 1956). The compound was made visible by spraying with 10% (w/v) ammonium molybdate solution. The migration of the sample was the same as that of authentic glucose. A second sample of the residue was treated by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and the visual spectrum of the resulting solution was determined. The unknown showed a maximum at 488 m μ , which was identical with that of glucose treated in the same manner.

A sample containing 44.8 mg. of sterol glucoside from smoke was shown to contain 14.1 mg. of glucose when analysed by the phenol-sulphuric acid procedure. This value is 103% of the theoretical for a monoglucoside. On the basis of this concentration $[\alpha]_D^{27} + 50.0^\circ$ (c 4.56 in water) was obtained. Shriner & Fuson (1957) gave $[\alpha]_D + 52.3^\circ$ for D-glucose and $[\alpha]_D + 47.7^\circ$ for hydrated glucose. The time of formation and microscopic appearance of the osazone from the hydrolysate of the glucoside at pH 6 was indistinguishable in these properties from those obtained with a known glucose solution. The osazone had m.p. 201° (D-glucosazone has m.p. 205°; Shriner & Fuson, 1957).

Catalytic hydrogenation of the sterols. The sterol from tobacco or smoke was reduced catalytically at 25° and atmospheric pressure with 10% palladium on charcoal (Matheson, Coleman and Bell) as the catalyst. The hydrogen number was 284. The reduced material was recrystallized from 95% (v/v) ethanol, yielding white glistening crystals. The stanol from smoke had m.p. 142°, and that from tobacco m.p. 141–142°, $[\alpha]_D^{26} + 24.7^\circ$ (c 4.0 in chloroform) (Found: C, 81.6, 81.3; H, 12.9, 12.9%).

Gas-chromatographic analysis of sterols. The sterol from tobacco and smoke, as well as the reduced sterol, was analysed by gas chromatography. The materials were chromatographed on a 1 m. glass column of diameter 0.25 in., packed with 2.7% (w/w) silicone SE-30 (General Electric Co.) on 70–100 mesh Gas Chrom P (Johns-Manville Co.). The instrument used was the Perkin-Elmer model 154D, with a flame ionization detector (Perkin-Elmer Corp.). The column was maintained at 223°. The flow rate of helium was 32 ml./min. Octacosane was used as an internal standard (Figs. 1 and 2).

Separation of the tobacco sterols. The tobacco sterol mixture was separated into the individual components by using a MicroTek 2500 gas-chromatographic unit equipped with dual columns and thermal-conductivity detector (MicroTek Instruments Inc., Baton Rouge, Louisiana, U.S.A.). Two U-shaped copper columns (45 in. $\times$ $\frac{3}{8}$ in.) packed with equal weights of 3% (w/w) silicone SE-30 adsorbed on 80–100 mesh Gas Chrom P were employed for this separation. The temperature of the columns was increased from 220° to 240° at a constant rate of 4°/min. Three fractions were collected. Each of the fractions was rechromatographed under the same conditions and the pure fractions collected from a number of runs were used for infrared-absorption and mass-spectral studies.

RESULTS AND DISCUSSION

A mixture of the D-glucosides of stigmasterol, sitosterol and campesterol has been isolated from a commercial blend of flue-cured, Burley, Maryland and Turkish tobaccos (0.044% of crude product on a dry-weight basis). A mixture of the same sterol glucosides was also isolated from the smoke of cigarettes manufactured from the same blend of tobaccos (1.59 mg./1000 cigarettes).

The presence of sterol glucosides in cigarette smoke was surprising because of the presumed low volatility of such compounds. We were able to sublime unchanged significant amounts of the material isolated from tobacco by a short-path distillation procedure at 250° and atmospheric pressure, though there was much decomposition of the residual material.

Physical and chemical data for the sterol glycoside isolated from both tobacco and cigarette smoke indicated that this material was similar to the partially characterized steroid glucoside isolated from flue-cured tobacco leaves by Dymicky & Stedman (1959b). Acid hydrolysis of this material resulted in the isolation of glucose in an amount indicating a monoglucoside for the sterol. The sterol portion of the molecule was reported by Dymicky & Stedman (1959b) to belong to the campestanol series. The physical and chemical data for the sterol obtained in the present study (Table 1) agreed with those reported by these authors. In particular, the infrared-absorption spectrum of the tobacco sterol showed the same relative intensities as were reported for the partially characterized sterol in the 972 and 960 cm.⁻¹ region.

Dymicky & Stedman (1959b) concluded that their material did not appear to be a mixture of known sterols since the melting points of combinations of known Δ^5 compounds derived from campestanol should be lower than that of the sterol from flue-cured leaves. Cahnmann (1956) reported, however, that neither the similarity of melting points and crystal forms nor the lack of melting-

Table 1. *Properties of the tobacco sterols and derivatives*

Experimental details are given in the text.

	M.p.	$[\alpha]_D$	C (%)	H (%)
Tobacco sterol glucoside	293–294° (decomp.)	–40.0°	73.0 72.8	10.5 10.4
Calculated for mixture of glucosides of stigmasterol (60%), β -sitosterol (25%) and campesterol (15%)	—	—	73.0	10.3
Tobacco sterol	143–144°	–43.4°	83.4 83.6	12.3 11.9
Calculated for mixture of stigmasterol (60%), β -sitosterol (25%) and campesterol (15%)	142°*	–43.7°	84.2	11.9
Tobacco stanol†	142°	+24.7°	81.6 81.3	12.9 12.9
Calculated for mixture of stigmastanol (85%) and campestanol (15%)	—	+24.7°	81.7	12.6

* Experimentally determined.

† The stanols recrystallized from ethanol contain 0.5 mole of water/mole (Georg, 1954).

Fig. 1. Gas chromatogram of sterols from tobacco and internal standard (broken line) octacosane. The sterols were chromatographed on Gas Chrom P (70–100 mesh), coated with 2.7% SE-30, at 223° and a helium flow-rate of 32 ml./min., by using a flame ionization detector. The relative retention times, based on that of octacosane, were 2.64, 2.89 and 3.37 for peaks A, B and C respectively; those for authentic campesterol, stigmasterol and β -sitosterol were 2.67, 2.91 and 3.37 respectively.

point depression is sufficient proof for the identity and purity of two compounds, particularly steroids.

We have shown by gas-chromatographic analysis (VandenHeuvel, Sweeley & Horning, 1960) that the sterol isolated from either tobacco glucoside or cigarette-smoke glucoside was a mixture of stigmasterol, sitosterol and campesterol (Fig. 1). Mass-spectroscopic examination of the three fractions collected from the gas-chromatographic separation of the tobacco sterol indicated that the parent mass of the first peak was 400 (campesterol), of the second peak 412 (stigmasterol) and of the third peak 414 (sitosterol). The infrared-absorption spectra of the individual fractions were identical with those of authentic samples of the corresponding sterols. A quantitative measurement of peak areas of the gas chromatogram showed that the amounts of these sterols were: stigmasterol, 60, sitosterol, 25, and campesterol, 15%. We prepared

a synthetic mixture of authentic samples of the three sterols, by using the β -isomer of sitosterol, in the same proportions as those in the tobacco mixture. The known mixture was recrystallized from ethanol to give white crystals, m.p. 142°. This compares favourably with the melting points obtained for the sterol isolated from tobacco or cigarette smoke.

Johnson, Brostie & Jensen (1957) determined stigmasterol in a mixture from the relative intensities of the maxima at 972 and 960 cm^{-1} . By the use of a modification of this method the amount of stigmasterol in the tobacco or cigarette smoke sterol mixture was estimated to be 60%. The infrared-absorption spectrum of the mixture of natural sterols isolated was identical with that obtained from a synthetic mixture of stigmasterol, sitosterol and campesterol (12:5:3, by wt.).

These findings were also corroborated by the catalytic hydrogenation of the isolated sterol from tobacco or cigarette smoke. The amount of hydrogen consumed was compatible for the composition of the mixture as: stigmasterol, 60%, sitosterol, 25%, and campesterol, 15%. Hydrogenation of both stigmasterol and sitosterol results in the formation of stigmastanol; campesterol is reduced to campestanol. Gas-chromatographic analysis of the reduced product from tobacco or cigarette smoke indicated the presence of two peaks corresponding to stigmastanol (sitostanol) and campestanol in amounts of 84 and 16% respectively (Fig. 2).

Wright *et al.* (1962), using the classical approach, established the presence of stigmasterol D-glucoside in a mixture of sterol glucosides of similar nature as the mixture reported in the present study. However, these authors presented only mass-spectral data obtained on a mixture in proposing the additional possible presence of the glucosides of what appeared to be β -sitosterol and campesterol.

Fig. 2. Gas chromatogram of the reduced sterols from tobacco and internal standard (broken line) octacosane. The stanols were chromatographed on Gas Chrom P (70–100 mesh), coated with 2.7% SE-30, at 223° and a helium flow-rate of 32 ml./min., by using a flame ionization detector. The relative retention times, based on that of octacosane, were 2.47 and 3.29 for peaks A and B respectively; those for authentic campestanol and sitostanol were 2.54 and 3.23 respectively.

Our techniques enabled us to separate, identify and establish the relative amounts of the compounds in the mixture with more confidence than could be obtained by using the classical techniques. Although the results obtained did not establish the exact isomeric assignment of the sitosterol, they are consistent with the β -isomer assignment. This conclusion agrees with reports (Georg, 1954) that indicate that the β -isomer is the predominant sitosterol in plants. We do not know how much, if any, of the γ - or other isomers are present, since the $C_{(20)}$ or $C_{(24)}$ optical isomers of sitosterol would not be expected to separate under the gas-chromatographic conditions employed.

SUMMARY

1. A sterol glycoside was isolated from tobacco and cigarette smoke which on acid hydrolysis yielded D-glucose and a sterol mixture.
2. Quantitative determination of the sugar in the aqueous hydrolysate indicated that the isolated material was a monoglucoside.
3. The sterol mixture consisted of stigmasterol, sitosterol and campesterol.

4. Gas-chromatographic analysis, infrared-absorption-spectra determination and catalytic hydrogenation established the amounts of stigmasterol, sitosterol and campesterol in the mixture as 60, 25 and 15% respectively.

The authors thank T. B. Walker, J. B. Williams and W. D. Galloway for technical assistance, and E. C. Sunas for infrared-absorption-spectra and Dr J. M. Ruth for mass-spectra determinations. We are grateful to Dr M. Wall of the Natural Products Laboratory, Research Triangle Institute, and Dr W. M. Stokes, Department of Medical Research, Providence College, for generous gifts of authentic campesterol from the Bergmann collection, and to Dr W. J. Humphlett of Distillation Products Industries for additional supplies of authentic campesterol.

REFERENCES

- Briggs, D. R., Garner, E. F., Montgomery, R. & Smith, F. (1956). *Analyt. Chem.* **28**, 1333.
- Cahnmann, H. J. (1956). *J. org. Chem.* **21**, 1412.
- Divekar, V. D., Ambaye, R. Y., Panse, T. B. & Khanolkar, V. R. (1961). *Proc. Indian Acad. Sci.* **54B**, 57.
- Dubois, M., Gilles, K. A., Hamilton, G. K., Rebers, P. A. & Smith, F. (1956). *Analyt. Chem.* **28**, 350.
- Dymicky, M. & Stedman, R. L. (1958). *Tob. Sci.* **2**, 99.
- Dymicky, M. & Stedman, R. L. (1959a). *Tob. Sci.* **3**, 179.
- Dymicky, M. & Stedman, R. L. (1959b). *Tob. Sci.* **3**, 4.
- Fieser, L. F. & Fieser, M. (1959). *Steroids*, p. 351. New York: Reinhold Publishing Corp.
- Georg, A. (ed.) (1954). *Elsevier's Encyclopaedia of Organic Chemistry*, **14S**, p. 1827s.
- Grossman, J. D. & Stedman, R. L. (1958). *Tob. Sci.* **2**, 115.
- Johnson, J. L., Brostie, R. F. & Jensen, A. O. (1957). *Analyt. Chem.* **29**, 468.
- Johnstone, R. A. W. & Plimmer, J. R. (1959). *Chem. Rev.* **59**, 885.
- Khanolkar, V. R., Panse, R. B. & Divekar, V. A. (1955). *Science*, **122**, 515.
- Kosak, A. R. & Swinehart, J. S. (1960). *J. org. Chem.* **25**, 222.
- Kosak, A. R., Swinehart, J. S., Taber, D. & Van Duuren, B. L. (1957). *Science*, **125**, 991.
- Rodgman, A., Cook, L. C. & Latimer, P. H. (1959). *Tob. Sci.* **3**, 125.
- Shriner, R. L. & Fuson, R. C. (1957). *The Systematic Identification of Organic Compounds*, 4th ed., p. 301. New York: John Wiley and Sons Inc.
- Swift, L. J. (1952). *J. Amer. chem. Soc.* **74**, 1099.
- VandenHeuvel, W. J., Sweeley, C. C. & Horning, E. C. (1960). *J. Amer. chem. Soc.* **82**, 3481.
- Wright, H. E., Burton, W. W. & Berry, R. C. (1962). *J. org. Chem.* **27**, 918.