[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, R. J. REYNOLDS TOBACCO CO.]

Flue-cured Tobacco. I. Isolation of Solanesol, an Unsaturated Alcohol

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RECEIVED JANUARY 30, 1956

A low-melting unsaturated alcohol has been isolated from flue-cured tobacco in quantities corresponding to 0.4% of the dry weight of the leaf. The compound has been isolated from tobacco in three different stages of treatment, indicating that the leaf content of this alcohol does not change significantly during the processing of tobacco. Structural studies have indicated that the alcohol is 3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaene-1-ol.

Although knowledge of the alkaloids, acids, carbohydrates, structural units (cellulose, lignin, etc.) and inorganic materials in tobacco is considerable, little is known concerning the neutral ether-soluble compounds of tobacco leaf.[1] We have found no reports of isolation of compounds in a pure state from the neutral ethereal extract although several mixtures of closely related compounds have been reported. A mixture of saturated hydrocarbons has been reported by Chibnall, *et al.*,[2] while sterols have been reported by Shmuk.[3] We were interested, therefore, in the isolation and identification of the neutral ether-soluble compounds of tobacco leaf.

Cigarette tobacco consists of four major types: flue-cured, burley, Maryland and Turkish tobaccos. This study has been concerned with flue-cured tobacco, which constitutes over 50% of the tobacco used in cigarette production in the United States.

Flue-cured tobacco was extracted with methanol and then with ether. The extracts were concentrated and the residues were combined. To the combined residues, ether was added and the water-soluble materials were removed by washing with water. The residue after removal of the ether was next purified by either (a) precipitation from acetone at $-27°$ or (b) partitioning between 90% methanol and hexane. The precipitate from acetone or the residue from concentration of the hexane extract was separated into numerous fractions by a series of chromatograms utilizing alumina, silicic acid and Florisil. One of the largest fractions was a white solid which we have called solanesol.

The infrared spectrum of solanesol was observed (Fig. 1). Absorption at 6.0 μ indicated the presence of unconjugated double bonds. The absorption at 10.0 μ indicated the hydroxyl group to be other than that of a saturated alcohol, suggesting the possibility of an allylic alcohol grouping in the molecule. Particularly striking was the similarity of the spectrum of solanesol in Fig. 1 with the infrared spectrum of farnesol.[4] This similarity of spectra suggested a number of probable structures for solanesol, several of which are I, II and III, where n is in the range of 6 to 8.

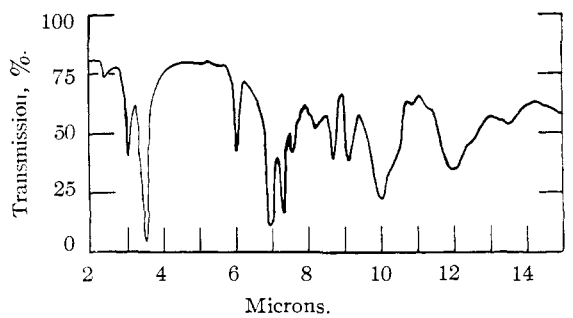
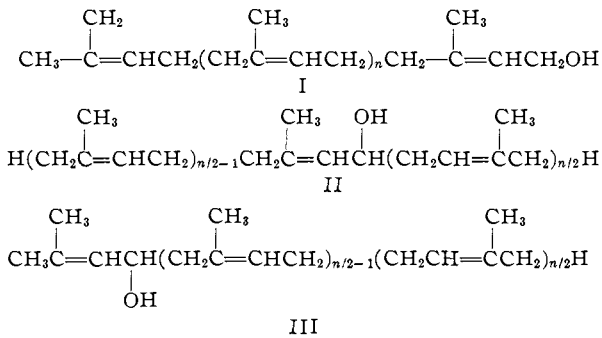


Fig. 1.—Infrared absorption spectrum of solanesol, oil.



I

II

III

Other positions of the hydroxyl group would also be possible and, moreover, instead of the chains being terminated with isopropylidene groups, the molecules could be terminated with ionone rings.

Quantitative catalytic reduction indicated slightly more than one double bond for each isoprene unit, 10.6 double bonds for I if $n = 8$. This high value in the double bond determination was the result of hydrogenolysis of the hydroxyl group. In one case, the yield of hydrocarbon was as high as 93%, but using other solvents and catalysts, the hydrocarbon and saturated alcohol were isolated in approximately equal amounts. Similar results were noted on catalytic reduction of farnesol.[5]

The infrared spectrum of the saturated alcohol obtained by catalytic reduction of solanesol showed that in the reduction the absorption due to COH had been shifted from 10 to 9.5 μ. A similar shift was observed upon comparing the spectra of farnesol, geraniol and phytol, all containing the grouping $-CH_2C(CH_3)=CHCH_2OH$, with the spectra of the corresponding reduction products. The hydrogenation experiments thus substantiated the presence in the molecule of the grouping $-CH_2C(CH_3)=CHCH(R)OH$ (IV). The nature of R in IV was determined by the oxidation of the saturated alcohol obtained from catalytic reduction of solanesol. The saturated alcohol $-CH_2CH-$

(1) W. G. Frankenburg in "Advances in Enzymology," Interscience Publishers, Inc., N. Y., 1950, Vol. 6, p. 309; Vol. 10, p. 325.

(2) A. C. Chibnall, S. H. Piper, A. Pollard, E. F. Williams and P. N. Sahai, *Biochem. J.*, **28**, 2189 (1934).

(3) A. Shmuk, *Vsesoyuz. Inst. Tabach. i. Makhoroch Prom.* No. **133**, 3 (1937); *C. A.*, **32**, 4723 (1938).

(4) J. Plina and F. Sorm, *Collection Czechoslov Chem. Communs.*, **14**, 247 (1949); *C. A.*, **44**, 935 (1950).

(5) I. M. Heilbron and A. Thompson, *J. Chem. Soc.*, 883 (1929); F. G. Fischer, *Ann.*, **464**, 69 (1928).

$(CH_3)CH_2CH(R)OH$ was readily oxidized by potassium permanganate in pyridine or in acetone to give a saturated acid, a result consistent with structure I, in which R = H, and inconsistent with structures II or III.

Quantitative determination of isopropylidene groups in solanesol indicated that the molecule contained only one isopropylidene group in agreement with structure I and contrary to structures II and III.

Structure I required one terminal methyl in each isoprene unit. A determination showed 0.72–0.73 terminal methyl group for each isoprene unit. Since the terminal methyl analyses usually show 0.8 or less for each group,[6] our results are consistent with structure I.

Of the structures considered for solanesol, only structure I would agree with the products isolated from ozonization experiments. From ozonization followed by reductive cleavage of the ozonide, levulinaldehyde and acetone were isolated as the 2,4-dinitrophenylhydrazones. From an ozonization of solanesol followed by oxidative cleavage of the ozonide, levulinic acid, oxalic acid and glycolic acid were isolated. The levulinic acid was identified by the infrared spectrum of its sodium salt and by conversion to the *p*-phenylphenacyl ester and to the 2,4-dinitrophenylhydrazone. Oxalic acid was precipitated as the calcium salt and was analyzed by oxidation with permanganate. Glycolic acid was identified by the infrared spectrum of its sodium salt and by conversion to its *p*-phenylphenacyl ester. Determination of the relative amounts of acids showed the equivalents of levulinic acid to be about seven times that of the combined equivalents of oxalic and glycolic acid.

Although the molecular weight determination of solanesol indicated that $n = 8$ in structure I, a value of $n = 6$ or 7 would agree with most of the other data which we have discussed, namely, elemental analyses, quantitative unsaturation analyses, terminal methyl determination and the products from ozonization. The most convincing evidence that n was 8 rather than 6 or 7 was related to the analyses of ester derivatives of solanesol. The acetate ester, 3,5-dinitrobenzoate ester, 3-nitrophthalate ester and *p*-phenylazobenzoate ester of solanesol were prepared. In each instance the analyses agreed well with the fact that n was 8.

The structure proposed for solanesol is similar to that of other natural products, *e.g.*, geraniol, nerol, farnesol, and yet it is surprising in two respects. Firstly, the acyclic triterpene squalene and the acyclic tetraterpenes lycopene and lycoxanthin possess symmetrical arrangement of the carbon skeletons. The cyclic tetraterpenes are also characterized by the symmetrical structure. It was unexpected, therefore, that the isoprene chain of solanesol would be unsymmetrical. The second surprising feature of solanesol is that it is a pentaterpene. Very little has been reported on the isolation of natural products containing ten isoprene units. An unsaturated hydrocarbon isolated from pig livers has been assigned a formula of $C_{45}H_{76}$ or $C_{50}H_{84}$ but the exact structure has not been reported.[7]

(6) P. Karrer, *Helv. Chim. Acta*, **13**, 1098 (1930).

Two major steps are involved in the preparation of tobacco prior to utilization in tobacco products. The first step is curing, a drying operation resulting in loss of large amounts of water and a change in leaf color from green to yellow or brown. Flue-curing is a rapid curing involving the use of artificial heat and elevated temperatures. The second major step is aging of tobacco. Flue-cured tobacco is packed in large wooden barrels called hogsheads to age for a period of 2 years before it is utilized in cigarette manufacture. During the curing and aging, considerable changes occur in the nature of the organic compounds present in the leaf. Accordingly it was of interest to determine the presence of solanesol at these different stages of processing. Since our isolation studies were not quantitative, a small change in amount was not detectable. However, considerable quantities of solanesol were found in the green leaf prior to curing, in the cured but unaged leaf, and in the aged tobacco. The material isolated from the green leaf amounted to 0.05% of the leaf, an amount considered to be 0.3% of the dry weight. From the unaged flue-cured tobacco, solanesol was isolated in an amount 0.4% of the leaf. From aged tobacco, the amount of solanesol isolated was 0.4% of the dry weight.[8]

Acknowledgments.—We are indebted to Mr. John J. Whalen for the infrared spectra and to Mr. R. H. Cundiff and Mr. B. N. Sullivan, Jr., for certain analytical determinations. We also wish to acknowledge the capable assistance of Mr. H. E. Moser.

Experimental[9]

Isolation of Solanesol. From Aged Flue-cured Tobacco.—Flue-cured tobacco, a blend of Government Grades B3L, B3F, C2F, H2F and H2L, grown in the "Old Belt," was aged for two years. Analyses showed 9.5% moisture, 21% sugars and 2.1% nicotine. Extraction of 30 kg. of shredded tobacco was accomplished by upward flow of 300 l. of methanol followed by 220 l. of ether through the tobacco packed in a stainless steel column. The extracts were concentrated. To the combined residues were added 16 l. of water. The mixture was extracted with two 12-l. portions of ether after which it was extracted with 52 l. of ether in a countercurrent extractor. The combined ethereal extracts were extracted with 20 l. of buffer solution (6 kg. of disodium phosphate heptahydrate and 1 kg. of potassium hydroxide in 20 l. of water, *p*H 11.8) using a countercurrent extractor. The ethereal solution was concentrated. The residue was partitioned between 40 l. of hexane and 40 l. of 90% methanol, again using the countercurrent extractor. The hexane layer was concentrated to a residue of 1015 g.

Chromatogram 1.—Using a 250 × 45 mm. (diam.) column of silicic acid, 12.0 g. of hexane-soluble material was chromatographed. The eluted fractions were concentrated under reduced pressure. The residual weights of the first five fractions are tabulated.

Chromatogram 2.—Fraction 4 of chromatogram 1 was applied to an alumina (Merck) column 180 × 45 mm. (diam.). The column was first treated with 900 ml. of 2:1 benzene–chloroform which eluted only 0.056 g. of material. Subsequently the solvent was changed to 1:2 benzene–

(7) H. J. Channon, J. Devine and J. V. Loach, *Biochem. J.*, **28**, 2012 (1934).

(8) Since our isolation of solanesol from flue-cured tobacco, it has also been isolated from Turkish tobacco by Dr. Gilbert Ashburn of these laboratories.

(9) All melting points were determined using a Fisher–Johns melting point apparatus. Elemental analyses were performed by the Clark Microanalytical Laboratory, Urbana, Ill.

Fraction	Volume, ml.	Solvent	Wt. of residue, g.
1	300	Hexane	0.6
2	300	CCl_4	0.8
3	80	Benzene	1.5
4	320	Benzene	2.9
5	400	Benzene	0.7

chloroform and the material (0.153 g.) eluted by the next 300 ml. was discarded. Solanesol (1.551 g.) was eluted in the following 900 ml. of solvent mixture. Following the solanesol a brown band (0.780 g.) was eluted with 300 ml. of 1:2 benzene–chloroform and 200 ml. of chloroform.

Purification was also effected by chromatography using 1:1 Celite[10]–Darco G-60[11] from which solanesol was eluted by hexane–carbon tetrachloride mixtures or by chromatography using Florisil[12] from which solanesol was eluted by benzene.

Isolation of Solanesol. From Unaged Flue-Cured Tobacco.—Flue-cured tobacco, 6.0 kg., Government Grades X4F and C2F, obtained from the Winston-Salem market, was not aged in the usual manner preparatory to cigarette manufacture but was kept at −27° for 1.5 years. It was then dried, powdered and extracted with 96 l. of methanol. The residue from the concentration of the methanol extract was mixed with 14 pounds of Celite[10]; the mixture was extracted with 70 l. of water after which it was extracted with 70 l. of methanol and with 20 l. of 1:1 methanol–ether. The methanol and methanol–ether extracts were concentrated to a residue of 191 g.[13] The residue was treated with 500 ml. of acetone. The insoluble material was removed by filtration and the filtrate was chilled to −27°. The precipitate was collected and was purified by chromatography on alumina and on silicic acid. In this manner 22 g. of solanesol was obtained, 11.5% of the water-insoluble methanol extract.

Isolation of Solanesol. Before Flue-curing.—Dixie Bright 101 (170 kg.), grown near Kernersville, N. C., was extracted on the day that it was picked. Extraction and purification were accomplished by the procedures described in the preceding two sections. Ultimate purification was accomplished by elution chromatography with alumina followed by chromatography from Florisil, giving 76 g. of solanesol.

The infrared spectrum was identical with the spectra of the materials isolated from the extraction of flue-cured and of aged flue-cured tobacco. This quantity corresponds to 0.045% of the weight of the tobacco. Since the green leaf ordinarily contains 80–85% water,[14] solanesol would constitute 0.22–0.3% of the dry weight of the uncured leaf.

Solanesol.—Solanesol is a waxy white solid melting at 41.5–42.5°. A slight yellow coloration was removed by crystallization from methanol, toluene or hexane at −27° or by chromatography using charcoal. In some cases, after chromatography from charcoal, solanesol decomposed on standing at room temperature. Solanesol is soluble in organic solvents but insoluble in water, exhibits no selective absorption in the ultraviolet and is optically inactive. *Anal.* Calcd. for $C_{50}H_{82}O$: C, 85.90; H, 11.80; mol. wt., 699. Found: C, 85.49, 85.98; H, 11.68; mol. wt. (ebull.), 654, 678.

Catalytic Hydrogenation.—Quantitative hydrogenation was accomplished in ethyl alcohol using palladium-on-charcoal catalyst at atmospheric pressure. Values of 68, 65 and 65 were obtained for the equivalent weight, indicating 10.3 to 10.8 double bonds for a molecular weight of 699.

Larger scale reduction (6.6 g.) of solanesol with palladium-on-charcoal in ethyl alcohol, followed by chromatography of the product with alumina, gave a 93% yield of saturated hydrocarbon. Reduction of 3.0 g. of solanesol with Pd–C catalyst in ether gave 1.47 g. of hydrocarbon and 1.59 g. of saturated alcohol.

Catalytic hydrogenation of 1.8 g. of solanesol was also accomplished in absolute alcohol using Adams catalyst at 2,000 pounds pressure. The oily residue (1.5 g.), obtained after removal of catalyst and solvent, was separated by chromatography on an alumina column into hydrocarbon and alcohol fractions. The hydrocarbon, 0.55 g., was a viscous oil, $n^{26}D$ 1.4582. *Anal.* Calcd. for $C_{50}H_{102}$: C, 85.4; H, 14.6; mol. wt., 702. Found: C, 85.5; H, 14.6; mol. wt. (ebull.), 623, 669. The reduced alcohol was eluted from alumina with chloroform. The alcohol was purified by chromatography with Florisil. The resulting material, 0.67 g., was optically inactive, $n^{26}D$ 1.4632. *Anal.* Calcd. for $C_{50}H_{102}O$: C, 83.48; H, 14.29; active H, 0.14. Found: C, 83.07; H, 13.91; active H, 0.16.

Oxidation of Perhydrosolanesol.—To a solution of 0.75 g. of the alcohol obtained by reduction of solanesol in 25 ml. of anhydrous pyridine was added 0.8 g. of powdered potassium permanganate.[15] The product was purified by chromatography with silicic acid, from which it was eluted by mixtures of carbon tetrachloride and benzene; yield 0.7 g. (90%), $n^{23}D$ 1.4633. *Anal.* Calcd. for $C_{50}H_{100}O_2$: C, 81.89; H, 13.74; mol. wt., 733. Found: C, 81.41; H, 13.53; mol. wt. (ebull.), 675, 694. The neutral equivalent was determined to be 697 by titration with tetrabutylammonium hydroxide in pyridine.[16]

Catalytic Reduction of Naturally occurring Allylic Alcohols.—Small samples (8 mg.) of geraniol, phytol[17] and farnesol[17] were each reduced in absolute alcohol solution with palladium-on-charcoal catalyst. Infrared spectra of the reduced alcohols showed absorption due to COH at 9.5 μ.

Terminal Methyl Determination.—Using the method of Barthel and LaForge,[18] equivalent weights of 95.7 and 97.5 were determined. From the calculated molecular weight of 699, the terminal methyl numbers of 7.3 and 7.2 were calculated.

Quantitative Determination of Isopropylidene Groups.—With farnesol, the procedure of Kuhn and Roth[19] showed 0.85, 0.90, 0.92 isopropylidene groups. With solanesol, the number of isopropylidene groups determined on the basis of a molecular weight of 699 was 0.89, 0.93, 0.93 and 0.98.

Reduction of the Ozonide of Solanesol. Isolation of Levulinaldehyde 2,4-Dinitrophenylhydrazone.—Following ozonization of 0.2 g. of solanesol in 75 ml. of ethyl acetate cooled in an ice–salt-bath, the ozonide was reduced with 0.2 g. of zinc dust and 100 ml. of water.[20] To the residue after steam distillation were added 160 ml. of 2 *N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The precipitate, after crystallization from nitrobenzene, melted at 232–233°. The infrared spectrum was identical with that of authentic levulinaldehyde 2,4-dinitrophenylhydrazone and a mixture of the two melted at 232–233°.

Reduction of the Ozonide of Solanesol. Isolation of Acetone as the 2,4-Dinitrophenylhydrazone.—The ozonide obtained from ozonization in chloroform at ice–salt-bath temperature was reduced with zinc dust. The volatile compounds from the reduction mixture were steam distilled into 300 ml. of 2 *N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The precipitate (Z) was collected, dried and extracted with hexane in a Soxhlet extractor. The hexane-insoluble material showed the spectrum of levulinaldehyde 2,4-dinitrophenylhydrazone. The hexane extract was concentrated and the residue was combined with the material extracted by chloroform from the filtrate of precipitate Z. The combined material was chromatographed on 2:1 silicic acid–Celite.[21] The acetone dinitrophenylhydrazone isolated from the chromatogram was recrystallized twice from ethanol, m.p. 124.5–125.5°. No depression of m.p. was noted with an authentic sample of acetone 2,4-dinitrophenylhydrazone; likewise, the infrared spectra of the authentic sample and that from ozonization were identical.

Oxidation of the Ozonide of Solanesol.—A mixture of 0.2 g. of solanesol and 75 ml. of ethyl acetate, cooled in an ice–salt-bath, was treated with ozone for 2.7 hours at a rate of 5 mmoles of ozone/hour. The ozonide obtained by concentration of the reaction mixture was oxidized with 3% hydrogen peroxide.[22] The reaction mixture, after treatment with

(10) A siliceous filter-aid produced by Johns-Manville Co., N. Y.

(11) Darco G-60, a product of the Darco Department, Atlas Powder Co., New York.

(12) Supplied by the Floridin Co., Warren, Pa.

(13) This extract, which was obtained in the course of other studies, was made available to us by Drs. S. A. Bellin and Gilbert Ashburn of these laboratories.

(14) W. W. Garner, "The Production of Tobacco," The Blakiston Co., Philadelphia, Pa., 1951, p. 399.

(15) L. Ruzicka and E. Rey, *Helv. Chim. Acta*, **24**, 529 (1941).

(16) R. H. Cundiff and P. C. Markunas, *Anal. Chem.*, **28**, 792 (1956).

(17) Obtained from Organic Research Chemicals, Bucks, England.

(18) W. F. Barthel and F. B. LaForge, *Ind. Eng. Chem., Anal. Ed.*, **16**, 434 (1944).

(19) R. Kuhn and H. Roth, *Ber.*, **65**, 1285 (1932).

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

(21) B. E. Gordon, *Anal. Chem.*, **23**, 1754 (1951).

(22) C. S. Marvel, W. M. Schilling, D. J. Shields, C. Bluestein, O. R. Irvin, P. G. Sheth and J. Honig, *J. Org. Chem.*, **16**, 838 (1951).

palladium–charcoal catalyst, was steam distilled. The distillation residue was studied by paper chromatography with 3:1 phenol:1% formic acid.[23] R_f values of 0.17, 0.56 and 0.91 were observed in agreement with those of glycolic, oxalic and levulinic acid. The ozonolysis was repeated a second time in identical fashion and the products from the two runs were combined.

The solution of non-volatile acids was concentrated to 5 ml. and was adjusted with 1 *N* sodium hydroxide to a *p*H of 5.5. One ml. of 25% calcium acetate solution was added and the precipitate was collected. Analysis of the precipitate[24] indicated that it contained 2.1 mg. of oxalic acid. The filtrate was adjusted to *p*H 4.0 with 0.5 *N* sulfuric acid and was concentrated to near dryness. The concentrate was chromatographed using 75 g. of silicic acid in a column of 50 mm. diameter and a mixture 4:1 chloroform–butanol saturated with 0.5 *N* sulfuric acid.[25] Each 25-ml. fraction was concentrated; the residue was dissolved in ethanol and was titrated to a phenol red end-point with sodium hydroxide. A major peak in elution of acids was noted in fractions 4–8 with a tailing into a minor peak in fractions 36–53. The fractions in the first peak, equivalent to 170 ml. of 0.018 *N* sodium hydroxide, were combined and concentrated to dryness. The infrared spectrum agreed with that of sodium levulinate. The *p*-phenylphenacyl ester was prepared.[26] After crystallization from benzene and from ethanol, it melted at 91–92° and showed the same infrared spectrum as that of an authentic sample. An authentic sample prepared from levulinic acid melted at 94–94.5°. The mixed m.p. of the two samples was 94–95°. *Anal.* Calcd. for $C_{19}H_{18}O_4$: C, 73.53; H, 5.85. Found: C, 73.22; H, 5.77.

The fractions from the minor second peak observed in chromatography with silicic acid were chromatographed again with 12 g. of silicic acid in a 20 mm. diam. column and 9-ml. fractions were collected. Peaks in acid content were noted in fractions 1–5 and in fractions 14–22. The residue from concentration of the fractions 1–5 was allowed to react with 2 *N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The precipitate, after crystallization from acetic acid, showed an infrared spectrum identical with that of levulinic acid dinitrophenylhydrazone.

Fractions 14–22, equivalent to 23 ml. of 0.018 *N* sodium hydroxide, were combined and concentrated. The residue was identified as sodium glycolate by infrared spectra. It was converted to the *p*-phenylphenacyl ester which exhibited an infrared spectrum identical with that of authentic *p*-phenylphenacyl glycolate. The authentic sample prepared from glycolic acid[26] melted at 122.5–123.5°. *Anal.* Calcd. for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.16; H, 5.43. The quantity of alkali required for the neutralization of levulinic acid in these titrations was *ca.* 7 times the quantity required for neutralization of the combined oxalic and glycolic acid.

Solanesol Acetate.—A mixture of 1.0 g. of solanesol, 10 ml. of chloroform, 0.14 ml. of pyridine and 0.2 ml. of acetic anhydride was heated under reflux for 3.5 hours. The mixture was diluted with ether and the ethereal solution was washed with 2 *N* sulfuric acid and with water. By chromatographic adsorption on 2:1 silicic acid–Celite, the product was separated into 0.5 g. of solanesol and 0.5 g. of solanesol acetate, m.p. 32–33°. Calcd. for $C_{53}H_{84}O_2$: C, 84.25; H, 11.42. Found: C, 84.12; H, 11.13.

Solanesol 3,5-Dinitrobenzoate.—The 3,5-dinitrobenzoate ester, prepared using benzene as the solvent,[27] was purified by chromatography on silicic acid followed by crystallization from acetone to give a solid melting at 57.5–59.5°. *Anal.* Calcd. for $C_{57}H_{84}N_2O_6$: C, 76.63; H, 9.48; N, 3.14; Found: C, 76.36; H, 9.46; N, 3.33.

Solanesol 3-Nitrophthalate.—The ester, obtained from 1.2 g. of solanesol,[28] was purified by chromatography on silicic acid followed by crystallization from acetone at −27°. The ester, 0.6 g., melted at 60–63° after sintering at 55°. *Anal.* Calcd. for $C_{55}H_{85}NO_6$: C, 78.07; H, 9.53; mol. wt. (neut. equiv.), 892. Found: C, 77.80; H, 9.38; mol. wt. (ebull.), 822; neut. equiv.,[16] 879.

Solanesol *p*-Phenylazobenzoate.—A mixture of 0.47 g. of solanesol, 50 ml. of anhyd. benzene, 0.4 g. of *p*-phenylazobenzoyl chloride and 4 drops of pyridine was heated under reflux for two hours. The product, after standard purification,[29] was extracted with 20 ml. of hexane and the hexane-soluble material was purified by chromatography on silicic acid. Only one colored ester band was noted during chromatography; it was eluted with 3:1 carbon tetrachloride–benzene; yield 0.33 g., m.p. 60–61°. Recrystallization from hexane at −27° did not alter the melting point. *Anal.* Calcd. for $C_{63}H_{90}N_2O_2$: C, 83.38; H, 10.00. Found: C, 83.19; H, 9.88.

(23) J. B. Stark, A. E. Goodban and H. S. Owens, *Anal. Chem.*, **23**, 413 (1951).

(24) G. W. Pucher, A. J. Wakeman and H. B. Vickery, *Ind. Eng. Chem., Anal. Ed.*, **13**, 244 (1941).

(25) R. Smeby, V. Zbinorsky, B. H. Burris and F. M. Strong, THIS JOURNAL, **76**, 6127 (1954).

(26) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 3rd Ed., p. 157.

(27) Reference 26, p. 164.

(28) Reference 26, p. 166.

(29) K. Ladenburg, E. Fernholz and E. S. Wallis, *J. Org. Chem.*, **3**, 294 (1938).

WINSTON-SALEM, N. C.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF LOUISVILLE]

2-Pyrones. XXII. β-Methylglutaconic Acid, β-Methylglutaconanilic Acids and Related Dianilides, Pyridones and Pyridazones

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RECEIVED MARCH 28, 1956

Decarboxylation of β-methylglutaconanilic acids (III) gives three types of products: 6-hydroxy-2-pyridones (IV), senecioanilides (XV) and 3-methyl-3-butenoanilides (XVI) indicating that the glutaconanilic acid reacts as an α,β-unsaturated acid. β-Methylglutaconic acid has been converted to dianilides (VII) and, *via* its anhydride, to γ-arylhydrazono derivatives XIV and pyridazonecarboxylic acids (XIII). A variety of 6-hydroxy-2-pyridones have been coupled with diazonium salts to give arylhydrazono derivatives V which have been converted to pyridazonecarboxylic acids. Dimethyl β-methylglutaconate undergoes coupling with diazonium salts to give mixtures of arylhydrazono derivatives XI and pyridazonecarboxylates XII, both of which have been converted to pyridazonecarboxylic acids and to arylhydrazonopyridazone carboxylates (X).

The discovery that β-methylglutaconic acid and several closely related structures are implicated in the coenzyme A catalyzed biosynthesis of cholesterol[1–3] and the suggestion[4] that the activity of α-phenylbutyramide in reducing blood cholesterol levels may result from its functioning as an antimetabolite in the coenzyme A catalyzed biosynthesis of cholesterol have established the desirability

(1) K. Block, L. C. Clark and I. Harary, *J. Biol. Chem.*, **211**, 687 (1954).

(2) H. Rudney, THIS JOURNAL, **76**, 2595 (1954); **77**, 1698 (1955).

(3) J. L. Rabinowitz, *ibid.*, **77**, 1295 (1955); **76**, 5168, 3037 (1954).

(4) J. Cottet and J. Redel, *La Presse Medicale*, **62**, 939 (June 16) 1954; *Compt. rend.*, **236**, 2553 (1953).