[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, THE HEBREW UNIVERSITY]

On the Oleuropein, the Bitter Principle of Olives[1]

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The bitter principle of olives, oleuropein (I), has been isolated in pure form from different parts of the olive tree. Crystalline ester and ether derivatives of I have been prepared. I is shown to be a double ester of glucose with protocatechuic acid (II) and with a new nonaromatic, unsaturated, optically active acid which is named oleuropeic acid (III). The constitution of III is established as 2,6-dimethyl-1-hydroxymethyl-1-carboxycyclohexene-2. The constitution of I, *i.e.* the points of linkage of the two acids to the sugar, have been determined.

The bitter principle of green olives was extracted by Vanzetti[2] from olive twigs as a levorotatory, amorphous substance. The grade of purity and the composition of the preparation were not ascertained.

Power and Tutin[3–5] prepared a bitter mixture of several amorphous compounds from olive leaves and bark which reduced Fehling's solution and gave a positive test with ferric chloride.

Bourquelot and Vintilesco[6,7] isolated the bitter principle from olives, olive leaves and olive bark. They described it as a bitter, noncrystalline glucoside, readily soluble in alcohol, fairly soluble in water and practically insoluble in ether. Its specific rotation was given as $-127°$ and its reducing power on Fehling's solution as 0.412 that of dextrose. They called it "oleuropein," a name which is accepted by us.

Hilts and Hollingshead[8] in a report of studies on the chemical changes during the ripening and pickling of olives suggested that the bitter principle is a tannin-like substance.

Cruess and Alsberg[9] confirmed that oleuropein has some common characteristics with tannin. Their preparations reduced potassium permanganate solution and were hydrolyzed both by acid and emulsin. It contained a double bond and phenolic groups. They concluded that the substance is a glucoside. They described caffeic acid as a component of the aglycone.

This report is concerned with the preparation of pure oleuropein (I) from different parts of the olive tree and the determination of its properties and its constitution.[10]

The preparations of I described by previous authors could be shown by chromatographic analysis to be mixtures of I with many other products. Methods were devised for isolation of pure I from various parts of the plant. The procedures include extraction with boiling acetone, precipitation with basic lead acetate, adsorption on charcoal, paper chromatography, and crystallization from ethyl acetate. Variations of the procedure did not change the nature of the final product which behaves chromatographically as a homogeneous substance. I thus prepared has m.p. 87–90° and the analytical composition and molecular weight corresponding to $C_{23}H_{30}O_{11}$. It reduces Fehling and ammoniacal silver nitrate solutions and gives the characteristical color reactions for an aromatic nucleus, phenolic groups, and sugar residues. The infrared spectrum shows bands (cm.$^{-1}$) at 3420 (free OH); 1710 (C_6H_5CO); 1640 (olefinic double bond); 1450 (—CH_2—); 1390, 920, 862 (—CHO); 1075 (—CH_2OH).

I could be extracted from green olives during summer months and from leaves, stems, and roots of the olive tree during the whole year. The concentration of I was found to be 0.6% of the dry material of the green fruit flesh and up to 6% of the cortex of the roots. The identity of I with the bitter principle of olives follows from the fact that it could be found only in the bitter green olives but not in the blackened ones which lack the bitter taste.

Several crystalline derivatives of I were prepared: 2,4-dinitrophenylosazone, tetraacetyl ester, tetrabenzoyl ester, monochlorotritosyl ester, dityrityl ether, and trimethyl ether.

The esters of I do not reduce Fehling or ammoniacal silver nitrate solutions and do not give a color reaction with ferric chloride. Their infrared spectra show the presence of an alcoholic group which cannot be esterified. The trityl ether is not reducing but still gives the ferric chloride reaction. The methyl ether neither reduces nor gives the ferric chloride reaction.

(1) This investigation had been supported by the Department of Commerce and Industry, Food Division, Israel. We thank Dr. J. Ilany-Feigenbaum, Director of the Food Division, for his aid and collaboration.

(2) B. L. Vanzetti, *Atti Accad. Lincei*, **2**, 189 (1908).

(3) F. B. Power and F. Tutin, *Pharm. J.*, **81**, 714 (1908).

(4) F. B. Power and F. Tutin, *J. Chem. Soc.*, **93**, 891 (1908).

(5) F. B. Power and F. Tutin, *J. Chem. Soc.*, **93**, 904 (1908).

(6) E. Bourquelot and J. Vintilesco, *Compt. rend.*, **147**, 533 (1908).

(7) E. Bourquelot and J. Vintilesco, *J. Pharm. Chim.*, 303 (1908).

(8) R. W. Hilts and R. S. Hollingshead, *U.S.D.A. Dept. Bull.*, 903 (1920).

(9) W. V. Cruess and C. L. Alsberg, *J. Am. Chem. Soc.*, **56**, 2115 (1934).

(10) Preliminary communications: B. Shasha and J. Leibowitz, *Bull. Res. Counc. of Israel*, **A8**, 92 (1959); Nature, **184**, 2019 (1959).

The assumption of the former authors that I is a glucoside and that the aglycone contains caffeic acid is disproved. I is not a glucoside but a double ester of glucose. The hydrolysis of I by emulsin could not be confirmed and must be attributed to impurities which are included in the crude preparations. The formation of an osazone clearly demonstrates the presence of a free glucosylic group and accidently also of a free hydroxyl at C_2 of the sugar moiety of the compound. Moreover, the crystalline preparations of I show mutarotation, a fact overlooked by the previous investigators. I is hydrolyzed not only by acids but also very easily by alkali, thus confirming that the linkage is not of a glucosidic but of an esteric nature.

The acid hydrolysis of I produces a mixture of a sugar with organic acids. The mixture could be resolved by chromatography. The sugar could be isolated and identified as glucose. The nonsugar moiety could be shown to be a mixture of two acids. One of them is a phenolic acid (II) and the other is a nonaromatic, nonsaturated acid (IV). Both acids are optically inactive.

The alkaline hydrolysis of I partially or even totally destroys the sugar but produces the same acid II and a nonaromatic, nonsaturated acid with strong levorotation (III). II and III or II and IV are the only nonsugar constituents of the hydrolyzates of I. No caffeic acid could be detected in the acid or alkaline hydrolyzate. The caffeic acid found by Cruess and Alsberg[9] in the hydrolyzate of their oleuropein must therefore be considered a constituent of an impurity, possibly the same which is responsible for the alleged hydrolysis by emulsin.

Acid II was chromatographically identified with protocatechuic acid. Acid III was isolated as a crystalline substance of the composition $C_{10}H_{16}O_3$, which could not be identified with any known compound and is named oleuropeic acid. The sum of the formulas of glucose ($C_6H_{12}O_6$), of protocatechuic acid ($C_7H_6O_4$), and of oleuropeic acid ($C_{10}H_{16}O_3$) corresponds exactly—after elimination of two molecules of water—with the formula $C_{23}H_{30}O_{11}$ which has been established for oleuropein.

The analytical composition of III corresponds to a terpenoid. The infrared absorption spectrum shows the presence of a carboxyl which could be quantitatively titrated, of an olefinic double bond, and of an alcoholic hydroxyl group. Nevertheless, the compound could neither be acetylated nor benzoylated. The failure of the attempts to esterify the alcoholic group indicates a steric hindrance. III shows all the characteristic reactions of the olefinic double bond which also shows up in the infrared spectrum. III could be catalytically hydrogenated to yield a saturated acid $C_{10}H_{18}O_3$ (V), which lacks optical activity. The infrared absorption spectrum of V shows the features of III with the exception of the band for the double bond. III is very alkali-resistent but heating with acid causes a cleavage of the molecule into methanol and a nonsaturated optically inactive acid, which differs analytically from III just by the elimination of methanol. It must be concluded that the compound contains a CH_2OH group and that the elimination of the latter abolishes the center of the asymmetry. This acid is identical with the nonaromatic, nonsaturated acid IV produced by the acid hydrolysis of I. The infrared absorption spectrum of IV lacks the bands for —OH. This fact explains the difference between the results of the acid and alkaline hydrolysis of I. While II is produced in both cases only the alkaline hydrolysis yields the original III which is included in the molecule of I, while acid hydrolysis converts III to IV.

Catalytic dehydrogenation of III with sulfur powder yields 2,6-dimethyl benzoic acid. This fact proves the presence of a six-membered ring with attached —COOH and two vicinal —CH_3 groups. It must be assumed that during this reaction the labile —CH_2OH is eliminated as methanol.

The position of the double bond is determined by the results of the ozonolysis: Although the reaction product could not be isolated in pure form, it clearly shows the presence of an aldehydic group and of a —$COCH_3$ group. The double bond must therefore be adjacent to a —CH_3 group.

The total of these findings clearly establishes the structure of III as 2,6-dimethyl-1-hydroxymethyl-1-carboxy cyclohexene-2. (Fig. 1).

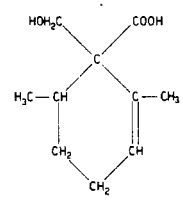


Fig. 1. Oleuropeic acid

Two features of this structure must be taken into consideration: 1) Although oleuropeic acid seems to be a terpenoid, it is not a diisoprene (Fig. 2). 2) Oleuropeic acid contains two asymmetric

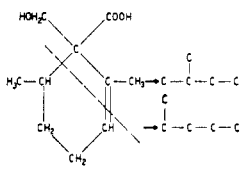


Fig. 2. Oleuropeic acid is a compound of one isoprene and one *n*-pentane chain

carbon atoms (C_1 and C_6). It is evident that the asymmetry of both is abolished by elimination of methanol, but the hydrogenation of the double bond should affect only the asymmetry of C_1 without destroying the asymmetry of C_6. The fact that compound V lacks optical activity could be explained by the assumption that the —$CHCH_3$

group in the oleuropein is originally racemic. This assumption can be strengthened by a precedent: Of the three naturally occurring mandelonitrile glucosides—prunasin, sambunigrin, and prulaurasin—the latter contains the aglycone in the D,L-form.[11,12] The analogy between the —CHCN group of the aglycone of the prulaurasin and the —$CHCH_3$ group of the nonsugar component of oleuropein is obvious. Another explanation for the lack of the optical activity in V could be the assumption that the hydrogenation over palladium catalyst has a directing effect on the addition of the two hydrogen atoms to the double bond,[13] thus producing only one configuration at C_2 which gives an inner compensation with the —$CHCH_3$ at C_6 (*meso* configuration). The problem whether V is a racemate or a *meso* compound is under investigation.

I, which is hydrolyzed neither by emulsin nor by yeast extracts containing maltase, is slowly attacked by extracts of *Aspergillus niger*, which contain tannase.[14] In the hydrolyzate, II, but not III, could be detected chromatographically.

The three components of oleuropein are linked together by ester bonds. Each of the five hydroxyl groups of the glucose could theoretically be esterified by each of the two acids. Another possibility of the structure of I is the depside type: the two acids linked together through esterification and the acid chain connected with an hydroxyl of the glucose by esterification.

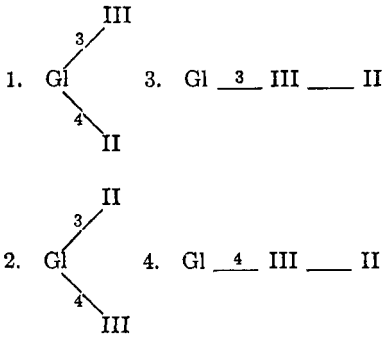
The mutarotation of I and the formation of an osazone exclude carbons C_1 and C_2 of the glucose from the possibility of participation in the ester formation. The characteristic reactions of polyphenols are evidence to the terminal position of II in the molecule, *i.e.* that the two phenolic hydroxyls are free. The hydrolysis of I by tannase, which liberates II but not III, confirms also the terminal position of II.

The three components of I contain altogether eight hydroxyls (five-glucose, two-II, one-III), two of which are necessarily occupied by the ester bonds. The acetylation and the benzoylation yield only tetraacyl derivatives which show in the infrared absorption spectra free hydroxyl groups which cannot be esterified. Therefore, a case of steric hindrance must be assumed. The investigation of III has shown that esterification of its alcoholic group is inhibited by the adjacent carboxyl. It can be assumed that this hydroxyl behaves similarly when III is included in I. The two phenolic hydroxyls are easily esterified or methylated, as neither the tetraacetate, tetrabenzoate, nor the tosylate or the methylate of I give phenolic reactions. Therefore, the second inactive hydroxyl of I must belong to the glucose.

Tritylation of I yields a ditrityl derivative which does not reduce Fehling's solution but still gives the color reaction with ferric chloride. As trityl is preferably linked to the primary alcoholic group[15] and as in the ditrityl oleuropein the reducing sugar group is absent, the two trityl residues must be attached to C_1 and to C_6, and the —CH_2OH group of the glucose in I must therefore be free.

The tritosyl monochloro derivative of I does not give reactions of the phenolic group, while only one of the three tosyl residues reacts with sodium iodide in a way which is specific for a tosylated primary alcoholic group.[16] Thus the tosyl residues are attached to the C_6 of the glucose and to the two hydroxyls of II, while the chlorine atom belongs to the glucosylic C_1-group of the glucose.

It follows that only the C_3 and C_4 groups of glucose remain as possible points of attachment for the acids. The number of possible structures of I is therefore reduced to four.



Using steric models, formulas 2 and 4 are immediately excluded, as the oleuropeic acid residue comes into collision with the —CH_2OH group of glucose. According to formulas 3 and 4, I should have four free hydroxyls in its glucose residue. Under this assumption there is no explanation for the formation of only tetraacyl derivatives of I which have only two acyl groups attached to the glucose residue. Moreover, under formula 4, the primary alcoholic group of III must be assumed to be esterified with the carboxyl group of II, contrary to the demonstrated inability of this alcoholic group to undergo esterification.

Formula 1 accounts for all experimental findings (Fig. 3). The steric model of this formula (Fig. 4) shows that the —OH of the C_2 of the glucose is hidden by the oleuropeic acid residue at C_3; thus its inactivity towards acetylation and benzoylation reagents and the formation of tetraacyl derivatives of I are easily explained.

(11) H. Hérissey, *J. Pharm. Chim.*, 24, 537 (1906).
(12) R. J. Caldwell and S. L. Courtauld, *J. Chem. Soc.*, 91, 666 (1907).
(13) K. N. Campbell and B. K. Campbell, *Chem. Revs.*, 31, 77 (1942).
(14) K. Freudenberg and E. Vollbrecht, *Z. physiol. Chem.*, 116, 277 (1921).
(15) D. D. Reynolds and W. L. Evans, *J. Am. Chem. Soc.*, 60, 2559 (1938).
(16) R. M. Hann, A. T. Ness, and C. S. Hudson, *J. Am. Chem. Soc.*, 66, 73 (1944).

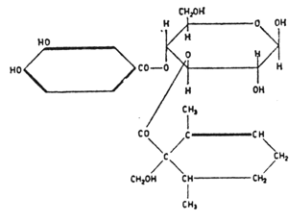


Fig. 3. The structure of oleuropein

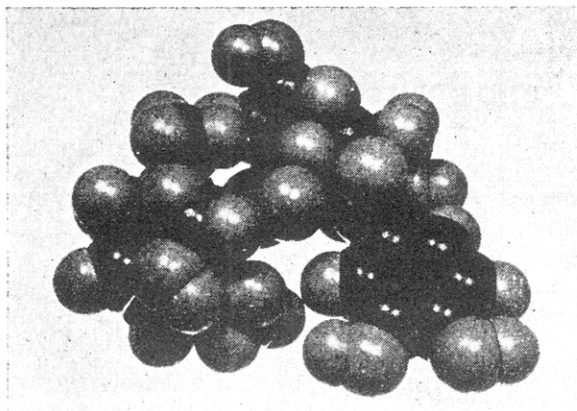


Fig. 4. The steric model of the oleuropein

Methylation of I yields a trimethyl derivative. This compound does not reduce Fehling's solution nor give a color reaction with ferric chloride. Therefore, the three methyl groups must have entered the reducing group of the glucose and the two phenolic groups of II. After acid hydrolysis the reducing power reappears thus confirming that one of the methoxyls was linked by a glycosidic bond.

In accordance with the formula I it is understandable that the C_2-group of the glucose withstands methylation. But no explanation can as yet be given for the nonmethylation of the $—CH_2$-OH group at C_6. Nevertheless, this one negative result cannot invalidate the proof of the constitution of oleuropein which is based on an accumulation of positive findings.

EXPERIMENTAL

Green fruits from the Palestine olive (*Olea europaea* var. *soori*) were collected for two years during the maturation period (July–December) and used fresh as a starting material for the isolation of the oleuropein.

Black olives from the same tree were used in parallel experiments. In a later stage of the work, leaves, roots, and stems of the same tree were used.

Preparation of crude oleuropein. Two kilograms of green olives were thoroughly washed with distilled water and then boiled with 3 l. of water for 2 min. After cooling the matter was ground by Waring blender in 100 g.-portions with 100 ml. of acetone for each portion. The acetonic suspension was heated at reflux for 2 hr. After cooling and centrifuging a clear yellow solution was obtained which is intensely bitter and shows strong levorotation. The residue was repeatedly extracted with boiling acetone until the extracts ceased to show optical activity.

The combined acetonic extracts (4.5 l.) were kept in a refrigerator for 3 days. A small quantity of long needle crystals were deposited which after filtration and recrystallization from ethanol proved to be mannitol[9]; m.p. 168°, $[\alpha]_D$ 0° (*c* 1, water), 25° (*c* 1, borax solution).

Anal. Calcd. for $C_6H_{14}O_6$: C, 39.56; H, 7.70. Found: C, 39.41; H, 7.70.

After elimination of the mannitol, the acetone solution was concentrated to a sirup which was redissolved in acetone. The insoluble material was eliminated by centrifuging and the process repeated for several times. After the fourth time a yellowish solid appeared during the concentration of the acetone solution (substance A). Chromatography shows that A is a mixture of several substances.

Purification of A by adsorption. A solution of 2 g. of A in 50 ml. water was shaken with 2 g. of charcoal (Mallinckrodt, New York) for 10 min. at a temperature of 50–55°. The suspension was filtered through a layer of 0.5 cm. of kieselguhr on a Buchner funnel. Elution was effected by sucking 25 ml. of ethanol (95%) through the charcoal-kieselguhr layer. A colorless solution was obtained from which after concentration *in vacuo*, solid agglomerates of an intensely bitter taste were deposited (substance I). For the chromatographic analysis of I, the organic phase of the mixtures butanol–acetic acid–water (4:1:5) or butanol saturated with water were used as solvents. As spraying agents 0.1% ferric chloride, ammoniacal silver nitrate, or diazotized sulfanilic acid solutions were used. The analysis showed the absolute homogeneity of the material: only one spot corresponding to Rf 0.73 (for the first solvent) or 0.42 (for the second) appeared.

Purification of A by paper chromatography. The chromatography was performed with an acetone solution of A on filter paper Whatman 3. Using the first solvent, the zone corresponding to the spot of Rf 0.73 was eluated with acetone. From the acetone solution a substance which is identical with I was obtained.

Purification of A by basic lead acetate. To a solution of 2 g. of A in 50 ml. of water, a solution of basic lead acetate was added until no further precipitation could be detected. The precipitate was centrifuged, washed with water, suspended in water and decomposed by hydrogen sulfide. The sulfide was filtered off and the clear solution concentrated *in vacuo* (40°), until dryness. The chromatographical analysis of the acetonic solution as above showed 2 distinct spots, one of which had R_f 0.73. This substance was dissolved in absolute alcohol, the insoluble material was eliminated by filtration and the solution concentrated. A substance identical with I was obtained.

Pure oleuropein. Substance I produced by either of the three methods mentioned above, could be recrystallized from ethyl acetate. The pure oleuropein has m.p. 87–90°. It is nitrogen-free.

Anal. Calcd. for $C_{23}H_{30}O_{11}$: C, 57.26; H, 6.25; mol. wt., 482. Found: C, 56.51; H, 6.90; mol. wt. 508 (camphor), 482 (water).

The substance is levorotatory: $[\alpha]_D$ −147° (*c* 1, water, methanol, ethanol or acetone) and shows mutarotation: $[\alpha]_D$ −127° after 9 hr. (*c* 1, water). It is very soluble in acetone, ethanol, methanol, pyridine, glacial acetic acid, and 5% sodium hydroxide. It is fairly soluble in dioxane, water, butanol, ethyl acetate, and butyl acetate. It is insoluble in ether, petroleum ether, chloroform, benzene, and carbon tetrachloride. It gives precipitates with neutral and basic lead acetate and is salted out from its water solution by ammonium sulfate or sodium chloride. It reduces Fehling's solution, potassium permanganate, and ammoniacal silver nitrate.

Qualitative color reactions of oleuropein. Oleuropein gives a dark-green color with ferric chloride. With concentrated sulfuric acid it gives a red color, with nitric acid a yellow-brown color; no color is produced by hydrochloric acid.

Formalin–sulfuric acid produces with a methanolic solution of oleuropein a light-violet color (test for an aromatic

nucleus).[17] Mitchell's reagent produces a greenish color which is not abolished by an excess of the reagent (test for catechol or pyrogallol groups).[18] 2,6-Dichloroquinone chloroimide gives with oleuropein a pink color which turned violet with borate buffer at *p*H 8–10 (test for phenols in which the *para* position is unoccupied).[19] Thymol-sulfuric acid produces a red color (a general reaction for sugars).[20] The color reactions clearly establish the presence of a sugar residue and of an aromatic nucleus with at least two *o*-phenolic groups.

Distribution of the oleuropein in the olive plant. The yield of oleuropein from green olives varied both in 1958 and in 1959 with the seasons. It was highest in June and declined steadily towards the end of the season (December). The highest yield of pure oleuropein was 1.8 g. from 2 kg. of fresh green olives. Taking into account that about 10% of the weight of the olives belongs to the stones and 17% to the oil and that the water content of the fresh fruit amounts to about 58%, the oleuropein represents about 0.6% of the dry material of the fruit flesh or 0.09% of the total fruit.

No oleuropein could be isolated from black olives during any period of the year.

Using the methods described above, oleuropein could be isolated from the fresh leaves, the roots, the branches, and the stem of the olive tree during all the seasons of the year. There were no appreciable seasonal variations in the amounts of oleuropein from the different parts of the plant, except from green fruits.

Both in the stem and in the roots all the oleuropein is concentrated in the cortex and no oleuropein could be detected in the pith. The yield of oleuropein from the root's cortex was the highest among all preparations from the different parts of the olive tree; it amounted to about 6% of the dry material. The crude preparations of oleuropein from the olive tree stems contain some phenyl benzoate which could be separated from the chief product by extraction with petroleum ether and isolated in pure form; m.p. 70°.

Anal. Calcd. for $C_{13}H_{10}O_2$: C, 78.77; H, 5.09. Found: C, 78.14; H, 4.78.

Derivatives of oleuropein. 2,4-Dinitrophenylosazone. Oleuropein gives well defined condensation products with aromatic hydrazines. The best result was obtained with 2,4-dinitrophenylhydrazine. Compound I (200 mg.) was dissolved in 3 ml. of alcohol and 15 ml. of 2,4-dinitrophenylhydrazine reagent was added. The solution was kept at 26°. After 2 days a red crystalline precipitate could be separated by filtration; m.p. 112°, $[\alpha]_D$ −113° (*c* 1 in alcohol-pyridine solution 4:6), after 22 hr. $[\alpha]_D$ −76°.

Anal. Calcd. for $C_{30}H_{37}O_{17}N_8$: C, 50.00; H, 4.40; N, 13.31. Found: C, 49.38; H, 4.35; N, 12.44.

The infrared absorption spectrum shows the characteristic bands of I supplemented by a band (cm.$^{-1}$) at 1340 (nitro group).

Acetylation. Compound I (200 mg.) was dissolved in 10 ml. of dry pyridine and 3 ml. of acetic anhydride. The solution was kept for 2 days at 25° and then poured into 50 ml. of distilled water. An oily layer appeared which was separated by decantation and crystallized from dilute alcohol. Recrystallization from absolute alcohol yielded microscopical crystals; m.p. 58–59°, $[\alpha]_D$ −62° (*c* 1, glacial acetic acid).

Anal. Calcd. for $C_{31}H_{38}O_{15}$: C, 57.23; H, 5.84; mol. wt., 682. Found: C, 56.41; H, 5.81; mol. wt., 663 (glacial acetic acid), 645 (camphor).

The determination of acetyl gave a value corresponding to four acetyl groups for 1 mole oleuropein. The tetraacetate is soluble in alcohol, acetic acid, ethyl acetate, acetone, and pyridine. It is insoluble in water, ether, hydrochloric acid, or sodium hydroxide solution. It does not react with ferric chloride or with ammoniacal silver nitrate.

Benzoylation. Compound I (800 mg.) was dissolved in 5 ml. of dry pyridine and heated at reflux with 2.5 ml. of benzoyl chloride for 20 min. at 75°. After cooling, 25 ml. water was added and an oily layer appeared which was separated by decantation. It crystallized from dilute alcohol (50%) and was recrystallized from absolute alcohol; m.p. 90–92°, $[\alpha]_D$ −84° (*c* 1, benzene), −72° (*c* 1, pyridine).

Anal. Calcd. for $C_{51}H_{46}O_{15}$: C, 68.14; H, 5.11; mol. wt., 898. Found: C, 67.70; H, 5.16; mol. wt., 926 (benzene).

The tetrabenzoyl derivative is soluble in benzene, glacial acetic acid, ether, acetone, and ethyl acetate. It is insoluble in water, petroleum ether, hydrochloric acid, and sodium hydroxide solution. It does not reduce Fehling's solution nor ammoniacal silver nitrate. It does not give a color reaction with ferric chloride.

Tosylation. Compound I (1.5 g.) with 1 g. of *p*-toluenesulfonyl chloride was dissolved in 5 ml. of pyridine and kept 24 hr. at 26°. The solution was poured into ice water. A sirup separated which solidified on scratching the inside of the vessel. The product was crystallized from dilute ethanol (50%); m.p. 83°. $[\alpha]_D$ −85° (*c* 1, methanol). This compound contains sulfur and chlorine.

Anal. Calcd. for $C_{44}H_{47}O_{17}S_3Cl$: C, 53.97; H, 4.71; mol. wt., 978. Found: C, 53.63; H, 4.75; mol. wt., 970 (benzene).

The analytical values and molecular weight correspond to a thrice tosylated and once chlorinated oleuropein.

This derivative is soluble in alcohol, acetone, benzene, acetic acid, and ether. It is insoluble in water, petroleum ether, dilute sodium hydroxide, and dilute hydrochloric acid.

The infrared absorption spectrum shows, besides the characteristic bands of I, bands (cm.$^{-1}$) at 1365, 1184 (O—SO_2).

Tritylation. Compound I (0.482 g.) was dissolved with 0.834 g. of trityl chloride in 10 ml. of dry pyridine. The solution was kept at room temperature. After 24 hr. colorless crystals appeared which were filtered and washed with water; m.p. 172°. $[\alpha]_D$ −38° (*c* 1, ethanol). The product contains crystal pyridine.

Anal. Calcd. for $C_{76}H_{73}O_{11}N_3$: C, 75.67; H, 6.06; N, 3.48; mol. wt., 1203. Found: C, 74.67; H, 5.99; N, 3.65; mol. wt., 1156 (camphor).

The analytical values and molecular weight correspond to a ditrityl derivative with 3 moles crystal pyridine.

This derivative does not reduce Fehling's solution but decolorizes potassium permanganate solution. It is soluble in ether, acetone, alcohol, dioxane, ethyl acetate, and glacial acetic acid. It is insoluble in water, petroleum ether, dilute sodium hydroxide, or dilute hydrochloric acid.

The infrared absorption spectrum shows the presence of all the functional groups which appeared in the oleuropein.

Methylation. Compound I (1 g.) was dissolved in a mixture of 10 ml. of methanol and 4 ml. of methyl iodide. Silver oxide, 1.5 g., was added and the suspension was shaken for 36 hr. at room temperature. The silver precipitate was filtered off and washed with methanol. The brownish filtrate is levorotatory. The solution was concentrated to one-third of its volume and poured into cold water. A sirup separated which was crystallized by scratching the inside of the vessel. After recrystallization from 50% methanol the substance was filtered and dried; m.p. 155–158°; $[\alpha]_D$ −92° (*c* 1, pyridine).

Anal. Calcd. for $C_{26}H_{36}O_{11}$: C, 59.54; H, 6.87; mol. wt., 524; methoxyl 17.7%. Found: C, 58.62; H, 6.84; mol. wt., 532 (camphor); methoxyl 17.94% (Zeisel's method).

The analytical values, the molecular weight and the methoxyl ratio correspond to a trimethyl derivative of I.

This derivative does not reduce Fehling's solution nor give a color reaction with ferric chloride.

The infrared absorption spectrum is practically identical with I because of the superposition of the bands for —OCH_3 and CH_2OH.

Acid hydrolysis of I. The oleuropein is readily hydrolyzed by boiling with organic and mineral acids (0.1 *N*), with velocities corresponding to the *p*K-values of the acids. The

(17)(a) L. Silverman and W. Bradshaw, *Anal. Chem.*, **27**, 96 (1955). (b) M. J. Rosen, *Anal. Chem.*, **27**, 111 (1955).

(18) C. A. Mitchell, *Analyst*, **48**, 2 (1923).

(19) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).

(20) Molish, *Monatsh.*, **7**, 198 (1886).

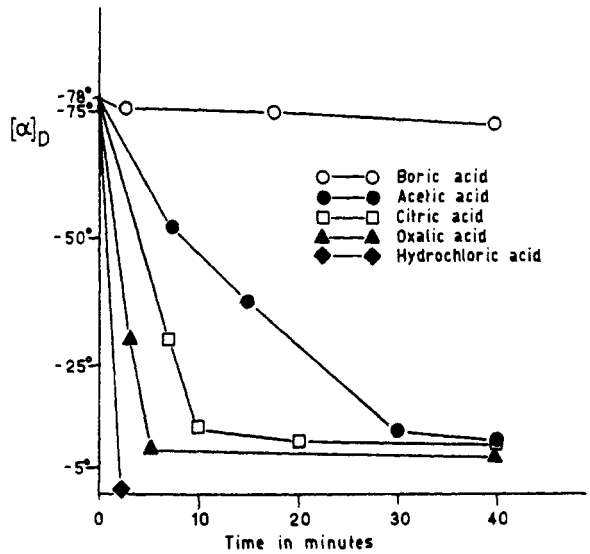


Fig. 5. Hydrolysis rates of oleuropein with different acids

hydrolysis could be followed by polarimetric observation. With the weak organic acids (acetic, oxalic, citric acids) a slight negative value of optical activity remains after hydrolysis; while hydrolysis with hydrochloric acid causes an inversion of the rotation from left to right.

From the hydrolyzate the sugar could be isolated and identified as glucose both by osazone formation and by chromatography. I, 2 g., was dissolved in 100 ml. of hydrochloric acid (*N*) and kept in a boiling water bath for 0.5 hr. After cooling sodium-acetate was added up to *p*H 5. Phenyl hydrazine, 4 ml., was added and the mixture was heated in a boiling water bath for 0.5 hr. Yellow crystals appeared which could be filtered, washed and dried; m.p. 207°; mixed m.p. with an authentic specimen of glucosazone was unchanged. The crystals were microscopically undistinguishable from authentic glucosazone.

The acid hydrolyzate was chromatographed on paper with butanol–acetic acid–water (4:1:5) as a solvent and with ammoniacal silver nitrate as a spraying agent. Two spots appeared, one of which was identical with the control spot of authentic glucose but different from control spots of mannose and fructose. The glucose in the hydrolyzate was quantitatively determined by the method of Somogyi-Nelson and amounted to 33% (109 γ glucose from 330 γ per ml. hydrolyzate). Calcd. for $C_{23}H_{30}O_{11}$: $C_6H_{12}O_6$ 37.4%.

The acid hydrolyzate of oleuropein was chromatographed on paper with butanol–acetic acid–water (4:1:5) or pyridine-butanol-water saturated with sodium chloride (1:1:2) as solvents. In both cases ammoniacal silver nitrate was used as a spraying agent. Caffeic acid and protocatechuic acid were used as controls. In both cases a spot appeared which was identical with the spot for protocatechuic acid. No spot corresponding to caffeic acid appeared.

In another experiment, the acid hydrolyzate of I was extracted with ether. The ethereal solution decolorized both potassium permanganate and iodine, thus demonstrating the presence of an olefinic double bond.

The alkaline hydrolysis of oleuropein. Compound I (5 g.) was dissolved in 20 ml. of sodium hydroxide solution (*N*) and heated in a boiling water bath for 10 min. The solution was cooled and neutralized against Congo Red (*p*H 4.5) by dilute hydrochloric acid. The solution was extracted with ether and gave a yellow levorotatory extract. The ethereal solution was slowly concentrated. During the concentration crystals appeared which could be filtered off leaving a sirupy residue which was chromatographically identified as protocatechuic acid.

The crystalline precipitate from the ethereal extract was recrystallized from water, dilute alcohol or absolute alcohol. Two forms of crystals were obtained (dimorphism): long needles at slow crystallization and lustrous plates at fast crystallization. Both forms show the same m.p. 158°, and the mixed m.p. remained unchanged. The crystals are colorless and tasteless. $[\alpha]_D$ −100°.

Anal. Calcd. for $C_{10}H_{16}O_3$: C, 65.22; H, 8.70; mol. wt., 184. Found: C, 65.67; H, 8.77; mol. wt., 196 (camphor).

This substance could not be identified with any compound described in the literature and has been named oleuropeic acid (III).

Oleuropeic acid. III is an acid which could be titrated with sodium hydroxide solution against phenolphthalein giving a value of one acid group per 1 mole of $C_{10}H_{16}O_3$ (C_9H_{15}-OCOOH). It shows the presence of an olefinic double bond by reaction with iodine and with potassium permanganate.

The infrared spectrum of III shows absorption bands (cm.$^{-1}$) at 3250 (bonded OH), 2810 (—CH_3), 2480, 1270 (—COOH), 1680, 1650 (olefinic double bond), 1075 (—CH_2-OH).

III does not reduce Fehling's solution or ammoniacal silver nitrate. It does not react with phenylhydrazine, substituted phenylhydrazines, semicarbazide, or hydroxylamine. It does not give a color reaction with ferric chloride. All attempts to obtain esters by acetylation with acetic anhydride–pyridine, or by benzoylation with benzoyl chloride–pyridine, failed; in all cases the unchanged substance was regained.

Determination of the double bond in III. Alcoholic solutions of III immediately decolorize alkaline potassium permanganate, with the production of brown manganese dioxide, and also decolorize bromine water. Quantitative determination of the double bond with Dam's reagent and potassium iodide gives the exact value of 1.

Catalytic hydrogenation of III. Compound III (390 mg.) was dissolved in 30 ml. of alcohol, 50 mg. of palladium (10%) was added, and hydrogen under the pressure of 60 p.s.i. was passed for 2 hr. through the suspension with shaking. After filtration the alcohol was evaporated. The sirupy residue crystallized on standing in the refrigerator and the product could be recrystallized from water. (Compound V).

Anal. Calcd. for $C_{10}H_{18}O_3$: C, 64.51; H, 9.67; mol. wt., 186. Analysis: C, 64.14; H, 9.73; mol. wt., 198 (camphor), 188 (titration with 0.1*N* sodium hydroxide).

V does not show any optical activity in organic solvents nor in sodium hydroxide solution (as sodium salt). It is soluble in ether, alcohol and dilute sodium carbonate or sodium hydroxide solutions. It is insoluble in water, benzene, chloroform, or petroleum ether. It does not reduce potassium permanganate.

The infrared absorption spectrum of V does not show the absorption bands of the olefinic double bond.

Benzylamine salt of III. Compound III (100 mg.) was added to 0.1 ml. of benzylamine and the mixture was heated in a small flask with reflux in a paraffin bath to 120° for 3 hr. After cooling, the material crystallized in needle-like crystals; m.p. 170°.

Anal. Calcd. for $C_{17}H_{25}O_3N$ ($C_{10}H_{16}O_3.C_7H_9N$): C, 70.01; H, 8.58; N, 4.82; mol. wt., 291. Found: C, 70.25; H, 8.09; N, 4.89; mol. wt., 298 (camphor).

The benzylamine salt of III is readily soluble in water and gives a positive ninhydrin reaction. It decolorizes alkaline potassium permanganate solution and bromine water.

Acid cleavage of III. Compound III (50 mg.) was added to 24 ml. of sulfuric acid (3.5*N*) in a small distilling flask, heated to 100° and distilled with a stream of steam. In the distillate methanol could be detected by the chromotropic acid test.[21] The distillate gave after cooling a colorless crystalline precipitate which could be recrystallized from dilute alcohol (compound IV); m.p. 132°.

Anal. Calcd. for $C_9H_{12}O_2$: C, 71.05; H, 7.89; mol. wt., 152. Found: C, 70.59; H, 8.45; mol. wt., 158 (titration with 0.1*N* sodium hydroxide).

(21) F. Feigl, *Spot Tests in Organic Analysis*, 5th ed., p. 339, Elsevier, New York (1956).

IV is insoluble in water but soluble in ether and alcohol. It is optically inactive both in organic solvents and in sodium hydroxide solution (as sodium salt).

The infrared absorption spectrum of compound IV does not show the band ($cm.^{-1}$) at 3250 (bonded OH).

Aromatization of III. A mixture of III (200 mg.) and 50 mg. of sulfur was heated in a small porcelain dish covered with a bigger one which contained ice water. The small dish was heated in a paraffin bath for 0.5 hr. at 180–250°. A strong odor of hydrogen sulfide was discernable immediately after the beginning of the heating. The production of hydrogen sulfide could be confirmed by lead acetate paper. From the small dish a substance sublimed and deposited as yellow crystals on the bottom of the big cooling dish. The crystals were dissolved in a solution of sodium hydroxide and reprecipitated by acidifying with hydrochloric acid. They could be extracted by ether, regained on evaporation of the solvent, and recrystallized from boiling water (compound VI); m.p. 112°.

Anal. Calcd. for $C_8H_{16}O_2$: C, 72.00; H, 6.66; mol. wt., 150. Found: C, 71.1; H, 6.78; mol. wt., 153 (titration with 0.1*N* sodium hydroxide).

VI is soluble in sodium carbonate or sodium bicarbonate solutions with the evolution of carbon dioxide; it is insoluble in cold water. Reactions with alkaline potassium permanganate or sodium nitroprusside solutions were negative. Methanolic, ethanolic or ether solutions of VI do not show any optical activity.

The amide of VI. A mixture of VI (18 mg.) with a few drops of twice redistilled thionyl chloride was heated at reflux for 30 min. at 70°. The excess thionyl chloride was distilled and 3 ml. of concd. ammonia was added to the residue which was again heated for several minutes. On cooling a crystalline precipitate appeared which could be filtered and recrystallized from 50% alcohol; m.p. 140°.

Anal. Calcd. for $C_9H_{11}ON$: C, 74.48; H, 7.58; N, 9.65. Found: C, 74.16; H, 7.03; N, 9.84.

By the analysis and m.p. of VI and of its amide, VI is identified as 2,6-dimethylbenzoic acid.[22, 23]

Ozonolysis of III. Compound III (50 mg.) was dissolved in a mixture of 10 ml. of glacial acetic acid and 3 ml. of acetic anhydride (for prevention of solidification of the acetic acid in the cold) in a small flask and immersed in an ice water bath. An ozone-oxygen mixture (1:10) from an ozonizer was passed through the solution. Zinc powder, 30 mg., was added and the mixture heated for 1 hr. on a boiling water-bath. The zinc was eliminated by filtration and the acetic acid and acetic anhydride by distilling *in vacuo*. The sirupy residue could not be crystallized, but it reduced Fehling's solution and gave positive nitroprusside and iodoform reactions. Iodoform crystals, m.p. 119°, could be isolated and gave an unchanged melting point after mixing with authentic iodoform.

Enzymatic hydrolysis of oleuropein. The oleuropein is attacked neither by emulsin which was very active in control experiments with amygdalin and salicin, nor by a preparation of α-glucosidase from baker yeast which was active in control experiments with maltose, nor by a lipase which was active on olive oil. Tannase, which was prepared from *Aspergillus niger* according to Freudenberg and Vollbrecht,[14] showed activity towards oleuropein at a temperature of 35° at *p*H 5. Evidence of hydrolysis was the liberation of protocatechuic acid which could be extracted and determined quantitatively by titration with sodium hydroxide solution (0.1*N*) against phenolphthalein. The amount of protocatechuic acid did not exceed 10% of the calculated amount for the total hydrolysis of oleuropein. No free oleuropeic acid could be detected in the hydrolyzate.

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(22) A. Shouberg and O. Kramer, *Ber.*, **55**, 1189 (1921).

(23) E. H. Rodd, *Chemistry of Carbon Compounds*, Vol. III, Part A, p. 544, Elsevier, New York (1954).

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Structures Related to Morphine. XVI.[1] Stereochemical Control of Addition of Hydrogen to 9-Oxobenzomorphans

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Hydrogenation of 2′-methoxy-2,5-dimethyl-9-oxo-6,7-benzomorphan methobromide (I) with platinum oxide has produced the carbinol methobromide II with the hydroxyl group oriented toward the nitrogen of the *cis*-fused *N*-containing ring as shown by infrared analysis and by its conversion to the *cis*-tetrahydrofurano compounds IV and VIII. Pyrolysis of II in boiling 1-nonanol gave the free base (VI). Similar hydrogenation of the base V yielded the diastereoisomeric carbinol (IX) to the apparent exclusion of VI; lithium aluminum hydride reduction of V afforded a 50–60% yield of IX and a 10% yield of VI. Treatment of VI and IX with boiling 48% hydrobromic acid led to the phenolic compounds X and XVII, respectively. Compound VI was also converted to the phenethyl analogs XV and XVIII which along with VI, IX, X, the di-*O*-acetyl derivative of X, and XVIII have been tested in mice for analgesic potential.

In the preceding paper[1] we reported that methylmagnesium iodide adds to 2′-methoxy-2,5-dimethyl-9-oxo-6,7-benzomorphan methiodide (I) to give a 9-methylcarbinol with the hydroxyl oriented toward the nitrogen (equatorial for the hydroaromatic ring), while the free base (V) and methyllithium or methylmagnesium iodide give principally the diastereoisomer. The present report is concerned with the addition of hydrogen to I and V to further study this perhaps partially electrically controlled asymmetric induction and to prepare compounds of possible neuropharmacologic value.

Hydrogenation of I using platinum oxide afforded the carbinol II with the hydroxyl *cis* to the iminoethano system analogous to the Grignard reaction.[1] This was proved by conversion of II in good yield to the furano compounds IV and VIII and by the fact that the infrared spectrum of the base VI, derived from II indicated strong OH—N bonding

(1) E. L. May and Hiroshi Kugita, *J. Org. Chem.*, **26**, 188 (1961).