

4-Bromo-6-(1,2-diphenylethyl)-7,8-dihydroxycoumarin (XXVI). One gram of 4-bromo-9-methoxypsoralene and 2.0 g. aluminum chloride were mixed intimately and then covered with 75 ml. benzene. This mixture was refluxed 20 min.; the benzene was then decanted and evaporated. The resulting residue was acidified with dilute hydrochloric acid, washed with water, and recrystallized twice from dilute ethanol and finally from dilute isopropyl alcohol; yield 0.46 g., m.p. 230–233°.

Anal. Calcd. for $C_{23}H_{17}O_4Br$: C, 63.3; H, 3.90. Found: C, 63.0; H, 4.07.

4-Bromo-7,8-dimethoxy-6-(1,2-diphenylethyl)coumarin (XXVII). 4-Bromo-7,8-dihydroxy-6-(1,2-diphenylethyl)coumarin (250 mg.) was dissolved in 20 ml. acetone. Two ml. dimethyl sulfate and one gram potassium carbonate were added and the solution was refluxed 17 hr. It was then poured into 100 ml. of water and cooled. The insoluble product was collected and recrystallized from dilute ethanol to yield 140 mg., m.p. 127–130°.

Anal. Calcd. for $C_{25}H_{21}O_4Br$: C, 64.5; H, 4.53. Found C, 64.3; H, 4.65.

3-[5-(2,3-Dihydro-6,7-dimethoxybenzofuryl)]propenoic acid (XXVIII). 2,3-Dihydro-9-methoxypsoralene (0.5 gram) was dissolved in 25 ml. acetone and 5.0 ml. of dimethyl sulfate was added. The solution was heated to reflux and 25 ml. of 20% potassium hydroxide was added slowly. Reflux was continued for 2 hr. after addition of the alkali. The solution was then cooled, acidified with dilute hydrochloric acid, and the acetone removed *in vacuo*. The insoluble product was collected and crystallized from dilute methanol to yield 0.3 g., m.p. 100–102°.

Anal. Calcd. for $C_{13}H_{14}O_5$: C, 62.5; H, 5.61. Found: C, 62.4; H, 5.72.

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CORVALLIS, ORE.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

The Functional Groups of Nomilin and Obacunone¹

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In conjunction with evidence derived from infrared spectra and including intensity measurements in the carbonyl-stretching region, the chemical properties of nomilin and obacunone indicate that these compounds are ketonic dilactones with a furan ring and but one carbocyclic system. One lactone ring of nomilin carries a β -acetoxy group whereas obacunone has the corresponding α,β -unsaturated lactone ring; this ring is readily opened by hydrolysis. The second lactone ring is opened by hydrogenolysis generating a carboxyl group which is remarkably acidic, properties that permit tentative conclusions to be drawn as to the relative positions of some of the functional groups.

Of the three optically active, lactonic, bitter principles of citrus fruits, limonin³ has been more thoroughly studied than nomilin and obacunone, knowledge of which is chiefly due to Emerson,⁴ who showed that both nomilin, $C_{28}H_{34}O_9$, and obacunone, $C_{26}H_{30}O_7$, gave obacunoic acid, $C_{26}H_{32}O_8$, on hydrolysis, the former yielding acetic acid at the same time. Further evidence indicated the presence of two lactone rings, one carrying a β -acetoxy group, and one of a somewhat unreactive carbonyl group. The present investigation has confirmed and extended these results.

The absence of hydroxyl groups from obacunone was clear from the inertness of this compound to acetylation and from its transparency near 3μ in the infrared. Because the intense and ill-resolved absorption (Fig. 1) in the region 1700–1734 cm^{-1} due to the lactonic system and carbonyl group made it difficult to detect individual functions, attention

TABLE I
ULTRAVIOLET END-ABSORPTIONS

	$\epsilon \times 10^{-3}$	
	209 $m\mu$	218 $m\mu$
Nomilin	5.70	4.27
Obacunone	16.7	16.0
Methyl obacunoate	13.6	11.3
Obacunone oxime	15.4	13.1
α -Obacunol	18.3	14.4
Dihydroobacunone	10.0	6.4
Obacunone hydrochloride	5.5	4.0
Double bonds ¹⁰		
(i) Disubstituted	~ 1	~ 0
(ii) Trisubstituted	3–4	~ 1.5
(iii) Tetrasubstituted	4.5–10	4–6
Furan	λ_{208} 5.6	λ_{216} 5.0
Marrubin ²⁹	λ_{210} 5.7	—

was directed to the spectrum of obacunone oxime, which, in paraffin mulls, had two bands of more or less equal intensity at 1749 and 1677 cm^{-1} . Whilst the latter band was correctly placed for the C=N stretching frequency of an oxime, its intensity was unexpectedly high compared with the data given by Cross and Rolfe,⁵ who quote oximes having extinction coefficients approximately one tenth those of ketones. In an attempt to minimize inter-

(5) L. H. Cross and A. C. Rolfe, *Trans. Faraday Soc.*, **47**, 354 (1951).

(1) This work formed part of a Technical Cooperation Project (1955) sponsored by the Foreign Operations Administration (U.S. Government).

(2) Department of Organic Chemistry, Liverpool University, Liverpool, England.

(3) O. H. Emerson, *J. Am. Chem. Soc.*, **74**, 688 (1952); B. V. Chandler and J. F. Kefford, *Australian J. Sci.*, **16**, 28 (1953); A. Fujita and Y. Hirose, *J. Pharm. Soc. Japan*, **76**, 129 (1956).

(4) O. H. Emerson, *J. Am. Chem. Soc.*, **70**, 545 (1948); **73**, 2621 (1951).

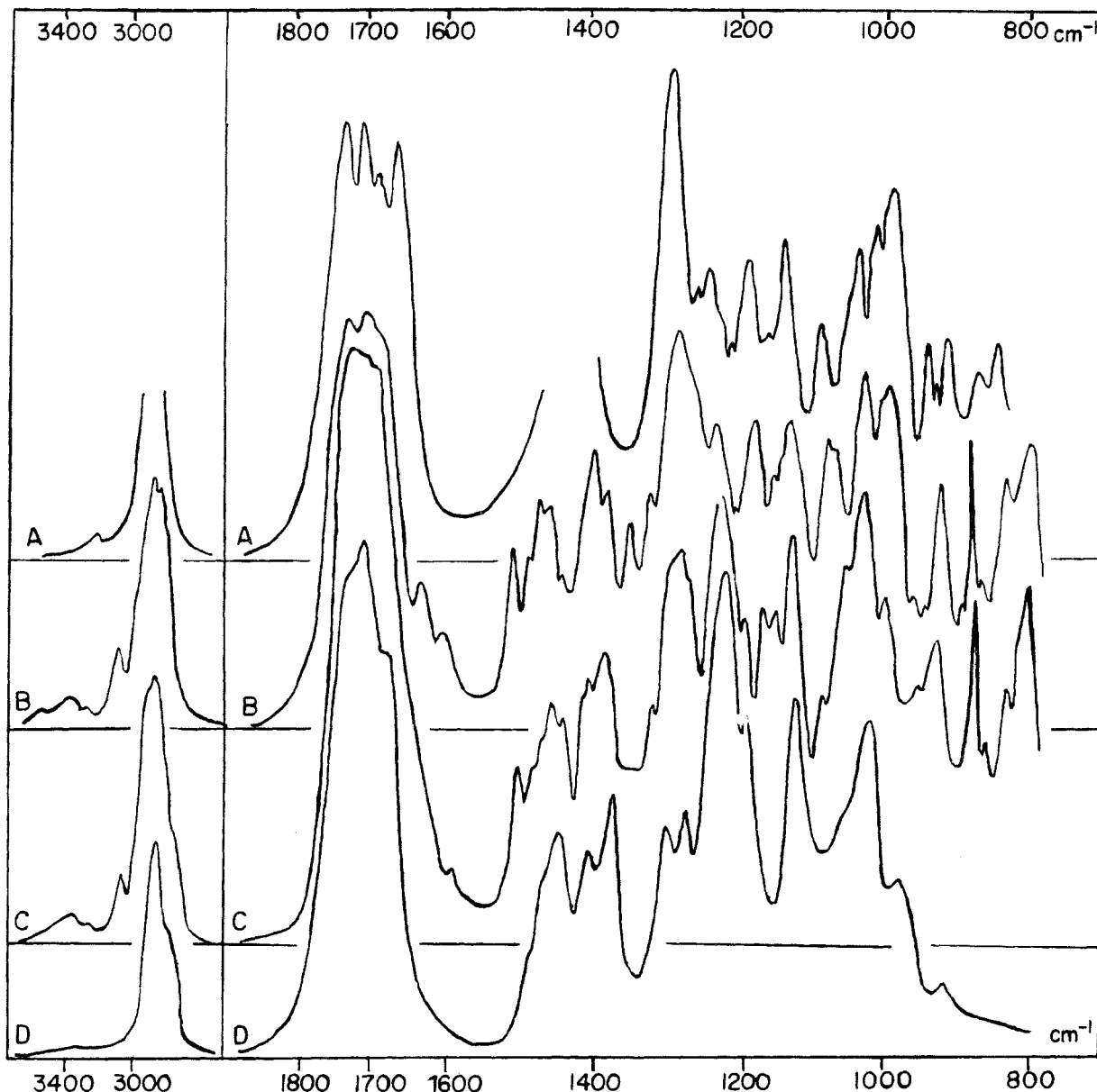


Fig. 1. A. Ozonolysis product of obacunone (in Nujol). B. Obacunone (film from CHCl_3). C. Nomilin (film from CHCl_3). D. Methyl hexahydronomilinate (film from CHCl_3)

ference from shifts of lactone bands induced by hydrogen-bonding, measurements were also made in chloroform, but in this solvent the oxime exhibited a complex band at $1694\text{--}1727\text{ cm}^{-1}$ and no marked absorption near 1670 cm^{-1} . This type of behavior does not appear to have been reported for simple oximes, and we have confirmed that the spectrum of cyclohexanone oxime is essentially the same in mulls and in solution. Pyruvic acid oxime had bands at 1700 cm^{-1} and 1660 cm^{-1} of the expected positions and relative intensities, but in parallel work complex changes were encountered when the spectra of *syn*- and *anti*-monoximes of benzil and camphorquinone were examined in mulls and in solution. On the other hand, the spectra of obacunone oxime were not in accord with the re-

sults obtained by Mathis⁶ for hydroxamic acids and indeed the substance gave no ferric reaction; but the possibility that hydroxylamine might have reacted with a lactone ring necessitated the acquisition of independent evidence for the presence of a carbonyl group.

The slow reaction between 2,4-dinitrophenylhydrazine sulphate and obacunone gave only amorphous material, but the rapid reaction with sodium borohydride gave a mixture consisting of a α -obacunol, $\text{C}_{27}\text{H}_{32}\text{O}_7$, with some of the isomeric β -obacunol. The presence of a third product was indicated by the intense color reaction given with hydrobromic acid, but the substance responsible

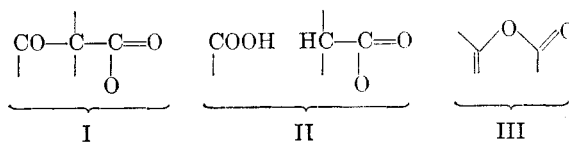
(6) F. Mathis, *Compt. rend.*, **232**, 505 (1951).

could not be isolated. Because borohydrides do not readily attack esters or lactones, α -obacunol was regarded as an alcohol derived from a ketone rather than a glycol from a lactone, a view confirmed by the formation of a monoacetate transparent at 3μ in the infrared. Attempts to re-oxidize α -obacunol to obacunone failed: Oppenauer's method did not appreciably affect the substance (a result which may have been due to steric hindrance, as the ketone is not very reactive or to complex formation),⁷ and chromic acid induced only ill-defined oxidation similar to that suffered by obacunone. Subsequently, the existence of a carbonyl group was clearly indicated by the spectra (discussed in the sequel) of related compounds having no ethylenic unsaturation but having well-resolved ester and lactone bands and also a band at 1711 cm.^{-1} typical of acyclic ketones or of cyclic ketones with more than five members. Although obacunone gave a positive iodoform reaction, an acetyl group is probably not present because the Zimmerman reaction⁸ was negative and because appropriate bands⁹ were absent from the infrared spectra. β -Obacunol has not been closely studied, but it may be diastereoisomeric with α -obacunol: both compounds had selective absorption at 3500 cm.^{-1} (OH), and at 1730 and 1690 cm.^{-1} (lactonic absorption).

The elimination of acetic acid from nomilin by means of boiling pyridine and acetic anhydride as described by Emerson⁴ gave erratic results not improved by omission of the acetic anhydride or by the addition of pyridine hydrochloride, but in α -picoline the reaction occurred regularly. Whilst this loss of acetoxy could not be followed from frequencies in the carbonyl-stretching region, bands typical of acetates⁹ and shown by nomilin at 1432 , 1377 , and 1225 cm.^{-1} were absent from, or much weakened in, the spectrum of obacunone. In agreement with previous work, removal of acetoxy resulted only in an increase in the ultra-violet end-absorption (Table I), which, taken with the fact that nomilin cannot contain a ketonic ring of less than six members, proved that obacunone was not an α,β -unsaturated ketone. This deduction was confirmed by the absence of marked bands near 1680 and 1640 cm.^{-1} or near 1720 and 1600 cm.^{-1} from the infrared spectrum. On the other hand, highly substituted double bonds¹⁰ have absorption characteristics near $210\text{ m}\mu$ closely simi-

lar to those of α,β -unsaturated esters¹¹ and lactones¹² so that the increase in this region could not be justifiably used to differentiate these systems, but a base-catalyzed as opposed to a thermal nature for the elimination was made probable not only by the stability of nomilin in xylene as opposed to γ -picoline but also by the ready loss of acetic acid accompanying the hydrolysis to obacunoic acid, and favored the formulation of obacunone as α,β -unsaturated lactone. Further, unconjugated double-bonds absorb near 1645 cm.^{-1} whereas (in this region) obacunone but not nomilin had a weak band at 1620 cm.^{-1} (Fig. 1), the position of which suggested conjugation: the corresponding bathochromic shift of a lactonic absorption was difficult to isolate from the complex band near 5.8μ .

As noted by Emerson, the ready hydrolysis of nomilin or of obacunone gave obacunoic acid, converted into the methyl ester by diazomethane. Acetylation of obacunoic acid gave Emerson only an amorphous solid of which the analysis seemed to differ little from that of the original substance; we have not been able to obtain an acetate of methyl obacunoate because in mild conditions no reaction took place, and in more vigorous conditions amorphous material resulted. In preliminary experiments, we were unable to confirm Emerson's comment (without experimental details) that methyl obacunoate possessed hydroxyl absorption near 3μ , so that when this ester was found not to react smoothly with hydroxylamine (in conditions under which obacunone formed an oxime comparatively readily) it became necessary to consider the possibility that the hydrolysis involved the fission of a (modified) β -ketoester system, as in (I) giving (II), rather than the fission of a lactone ring.



During attempts to obtain further information on this point, obacunoic acid and its methyl ester were subjected to sodium borohydride reduction, and α -obacunol was subjected to hydrolysis. In each case a rapid reaction ensued but the products were amorphous. In spite of its ill-defined nature, this evidence suggested that the carbonyl group was not involved in the hydrolysis and prompted a renewed search for hydroxyl absorption in the infrared spectrum of methyl obacunoate. When examined in paraffin mulls, in chloroform, or in carbon disulphide, this ester showed no absorption near 3μ sufficiently well-defined to be differentiated from overtones of the strong absorption near 6μ , but, surprisingly, solid films deposited from chloro-

(7) M. Ehrenstein, A. R. Johnson, P. C. Olmstead, V. I. Vivian, and M. A. Wagner, *J. Org. Chem.*, **15**, 264 (1950).

(8) W. Zimmerman, *Z. physiol. Chem.*, **233**, 257 (1935); I. E. Broadbent and W. Klyne, *Biochem. J.*, **56**, XXX (1954).

(9) A. R. H. Cole and D. W. Thornton, *J. Chem. Soc.*, 1007 (1956); R. N. Jones and A. R. H. Cole, *J. Am. Chem. Soc.*, **74**, 5648 (1952).

(10) P. Bladon, H. B. Henbest, and G. W. Wood, *J. Chem. Soc.*, 2737 (1952)

(11) H. E. Ungnade and I. Ortega, *J. Am. Chem. Soc.*, **73**, 1564 (1951).

(12) L. Dorfmann, *Chem. Revs.*, **53**, 47 (1953).

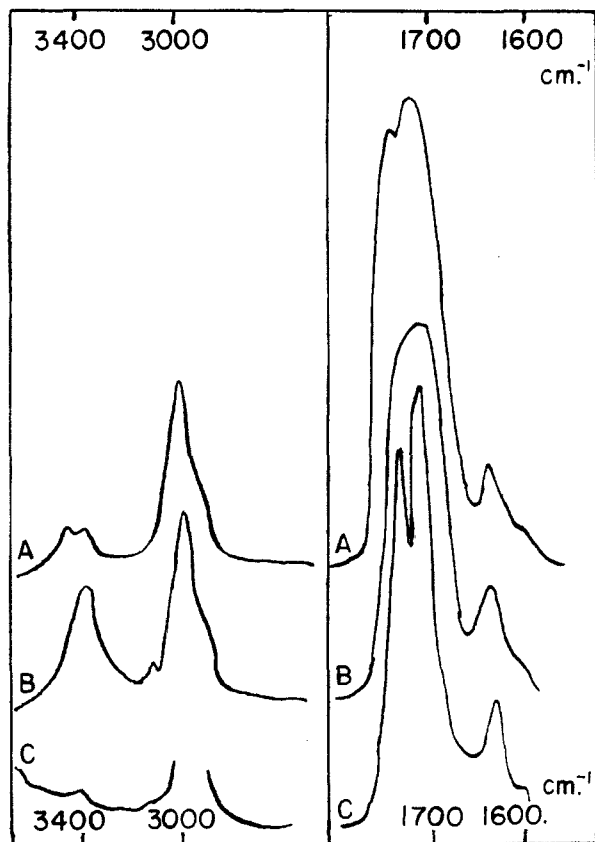


Fig. 2. Methyl obacunoate: A, in CHCl_3 . B, Solid film. C, Nujol mull

form or acetone showed a marked hydroxyl band (Fig. 2) which persisted when care was taken to exclude moisture and alcohol and is, therefore, positive evidence for the existence of an unreactive hydroxyl group. No other compound examined in this work behaved thus, but a somewhat similar phenomenon has been observed with tetric¹³ acids in which the degree of enolization and consequently the existence of hydroxyl or carbonyl groups depended upon the physical state of the sample. This explanation was apparently not applicable because methyl obacunoate did not have the requisite properties in the ultraviolet region, was not acidic, could not be oximated readily, and gave no ferric reaction. Further, had nomilin contained a lactonic system of type (III), hydrolysis would have yielded a keto-acid rather than a hydroxy-acid, but the parent compound must have exhibited absorption at 1770–1790 cm^{-1} which was not apparent. Finally, lactones of type (III) react readily with aniline¹⁴ whereas obacunone was recovered from treatment with this reagent.

Hydrogenation of nomilin was erratic unless large amounts of catalyst were employed, but in the best runs exactly four equivalents of hydrogen were absorbed. The product, hexahydronomilinic acid,

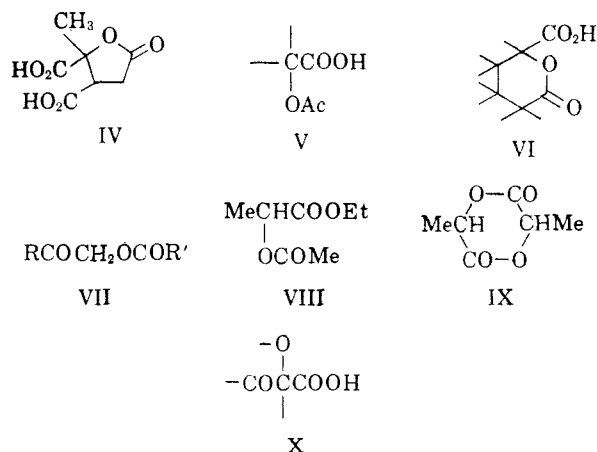
(13) L. A. Duncanson, *J. Chem. Soc.*, 1207 (1953).

(14) F. A. Kuehl, Jr., R. P. Linstead, and B. A. Orkin, *J. Chem. Soc.*, 2213 (1950).

$\text{C}_{28}\text{H}_{42}\text{O}_9$, was a monobasic acid of pK_a value 2.7, which appeared to have resulted from hydrogenolysis of a lactone ring not involved in hydrolysis since obacunoic acid had a pK_a value of 4.0. With diazomethane, the new acid gave a methyl ester spectroscopically devoid of hydroxyl groups but possessing a carbonyl group responsible for a shoulder at 1700 cm^{-1} on the ester band (Fig. 1). It was concluded that the four moles of hydrogen were used to open one lactone ring and saturate three double bonds since hexahydronomilinic acid could not be hydrogenated further and, unlike nomilin, gave no yellow color with tetranitromethane and had very little end-absorption at 210 $\text{m}\mu$. It followed that nomilin contained but one carbocyclic system.

The absence of double bonds from hexahydronomilinic acid made it necessary to suppose that the high acidity was due to the inductive effect of substituents at the α -position. Pyruvic acid having¹⁵ a pK_a value of 2.5, nomilin and its derivatives were first examined for α -ketoacid functions, but obacunone would not react with *o*-phenylenediamine under conditions in which pyruvates furnish quinoxalones. It has been reported¹⁶ that pyruvates have only unresolved ester absorption at 1745 cm^{-1} , whereas the carbonyl frequency is resolved in some compounds of the present series, and nomilinic acid was recovered from treatment of its salts with aqueous hydrogen peroxide.

The dissociation data given by Paul¹⁷ for α -acetoxypropionic acid (pK_a 3.0) and by Walden¹⁸ for the acid (IV) (pK_a 2.18) suggested that the acidity of hexahydronomilinic acid could have resulted from the groupings (V), found for example, in scilliroside,¹⁹ or (VI) as in monocrotalic acid.²⁰



(15) J. Boeseken, L. W. Hanson, and S. H. Bertram, *Rec. trav. chim.*, **35**, 309 (1915).

(16) References and a discussion of the spectra of coumarandiones are given by J. F. Grove, *J. Chem. Soc.*, 883 (1951).

(17) T. Paul, *Z. Elektrochem.*, **28**, 435 (1922).

(18) P. Walden, *Zeit. phys. Chem.*, **10**, 563, 638 (1892).

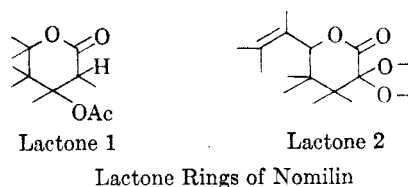
(19) A. Stoll and J. Renz, *Helv. Chim. Acta*, **25**, 377 (1942).

(20) R. Adams and T. R. Govindachari, *J. Am. Chem. Soc.*, **72**, 158 (1950).

The complex hydrogenation of obacunone was accomplished by methanolysis and gave an acid now called methyl hydrogen octahydroobacunonate since it was more easily obtainable by the comparatively smooth hydrogenation of methyl obacunoate in which nearly five moles of hydrogen were absorbed. The new acid had pK_a 2.9 for which neither system (V) nor system (VI) could be responsible. Systems (V) and (VI) are similar to α -acyloxyketones (VII) in which considerable shifts to shorter wave lengths of both carbonyl and ester frequencies occur,²¹ but we found that the corresponding shift in ethyl α -acetoxypropionate (VIII) was relatively slight and similar to that in mesodilactide²² (IX). This shift resulted in peaks at 1736 cm.^{-1} in chloroform and 1767 cm.^{-1} in carbon tetrachloride, but the appropriate derivatives of nomilin were hardly soluble in the latter solvent and so this shift could not be used as a criterion in the present example.

The possibility that the acidity of hexahydronomilinic acid was partly caused by a carbonyl group in the β -position was considered improbable because properties in keeping with β -ketoester and β -ketoacid systems were not apparent. For example, hexahydronomilinic acid did not readily lose carbon dioxide until decomposition set in at temperature greater than 220° . The presence of a malonic acid grouping was also discounted because this would have required the carboxyl groups of both hexahydronomilinic acid and obacunoic acid to have closely similar properties. Further, prominent shifts to shorter wavelengths are often shown by dilactones and related compounds with both carboxyl groups attached to the same carbon atom.²³ The composite system (X), in which combined inductive effects of carbonyl and of ether oxygen could have accounted satisfactorily for the acidity of the carboxyl group, was eliminated from further consideration because of the argument presented above and because these compounds had only very feeble reducing powers. Because methoxyacetic acid²⁴ has a pK_a value of 3.53 compared with 4.74 for acetic acid; two ether oxygen atoms would have an effect of the obscured magnitude, and it was confirmed that ethyl diethoxyacetate has a normal ester frequency at 1749 cm.^{-1} . Published data on the glycidic acid grouping are limited but it seemed unlikely that this system would survive prolonged hydrogenation, and consideration of data supplied by Kil-

patrick and Morse,²⁵ by Wode,²⁶ and by Apichandari and Jatkar²⁷ suggested that incorporation of the α -oxygen atom in a three-membered ring would not have any marked effect on the acidity.^{27a} Therefore the lactone rings of nomilin were provisionally written as in the diagram, which makes it clear that the lactone ring (called lactone 1) which becomes unsaturated in obacunone must also be that involved in the hydrolysis to obacunoic acid. The double bond attached to lactone 2 was required to account for the hydrogenolysis of this system.



A study of the intensities of carbonyl absorption in the 6μ region substantiated the conclusions reached above. As we were not aware of any published study of the intensities of lactonic absorption, we first made measurements on a few lactones typical of various types and collected the results into Table II; whereas Table III was adapted chiefly from values given by Jones, Ramsay, Kier, and Dobriner²⁸ for ketones and simple esters, except that the provisional nature of the present work made it unnecessary to include the minor variations detailed by these authors. Comparison of Tables II and III showed that those environmental changes which affect ketonic carbonyl affect ester carbonyl similarly. Thus the inclusion of either type of carbonyl group in a ring-system increased the intensity (the size of the ring being comparatively unimportant), and conjugation also increased the

TABLE II
INTENSITIES^a OF LACTONE CARBONYL ABSORPTIONS

	1×10^{-4}	Mean
γ -Valerolactone	4.12	3.92
Dihydrocoumarin	3.84	
Δ^2 -Angelicalactone	3.88	
Santonin (at 1775 cm.^{-1})	3.82	
Δ^1 -Angelicalactone	5.28	5.35
Coumarin	5.42	
Ethyl cinnamate	3.83	

^a Determined in chloroform by method III, Ref. 28.

(25) M. Kilpatrick and J. G. Morse, *J. Am. Chem. Soc.*, **75**, 1846, 1854 (1953).

(26) G. Wode, *Svensk. kem. Tidsk.*, **40**, 221 (1928).

(27) C. T. Apichandari and S. K. K. Jatkar, *J. Indian Inst. Sci.*, **21a**, 373 (1938).

(27a) Since glycidic acids (e.g. phenylglycidic acid) have now been found to have pK_a values near 3.6 in water and near 4.4 in aqueous acetone, these acids are less affected by solvent changes than benzoic acid. Consequently a glycidic acid system is still possible in the present series.

(28) R. N. Jones, D. A. Ramsay, D. S. Kier, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 80 (1952).

(21) R. N. Jones, P. Humphries, and K. Dobriner, *J. Am. Chem. Soc.*, **72**, 956 (1950); R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 2820 (1952); J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 877 (1951).

(22) H. H. Wasserman and H. E. Zimmerman, *J. Am. Chem. Soc.*, **72**, 5787 (1950).

(23) References and examples are quoted by D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 142 (1956).

(24) M. H. Palomaa, *Ann. Acad. Sci. Fennicae*, **A**, **1**, (1911); *Chem. Zent.*, **II**, 596 (1912).

intensity to some extent. Much the greatest increase resulted, however, when the carbonyl groups were simultaneously part of a ring system and conjugated with a double bond, thus permitting the easy differentiation of these structural types from others with similar ultraviolet spectra.

TABLE III
INTENSITIES^a OF CARBONYL ABSORPTION OF KETONES AND ESTERS

	1×10^{-4}
Acyclic ketones	1.8
Cyclic ketones	
(i) 7-, 11- or 12-ketosteroids	2.2
(ii) 3-, 16- or 17-ketosteroids	2.7
Cyclic $\Delta^{\alpha,\beta}$ -ketones	3.7
Cyclic $\Delta^{\beta,\gamma}$ -ketones	2.9
Alkyl acetates	3.2
Aryl acetates	2.8
Methyl esters	3.1
1-Acetylcyclohexene ^b	2.04

^a Adapted from values given in Ref. 28. ^b Determined in present work.

had opened. Finally, the result for α -obacunol was compatible with the reduction of a carbonyl group but not with the reduction of a lactone group.

As mentioned earlier, the results of hydrogenation showed that nomilin contained three relatively unreactive double bonds but both ultraviolet and infrared spectra were incompatible with the presence of a benzene ring. The fourth double bond of obacunone was more reactive and limited hydrogenation produced dihydroobacunone which had no significant absorption at 1620 cm.^{-1} , had a much reduced absorption below $220 \text{ m}\mu$ (Table I), and a carbonyl intensity which agreed with saturation of the lactone ring. When conducted in methanol, a similar hydrogenation gave a product which retained the solvent strongly in addition to two extra hydrogen atoms and had hydroxylic absorption near 3μ ; this compound may be the corresponding methyl dihydroobacunoate. Saturation of the lactone ring of obacunone resulted in the expected hypsochromic shift of the corresponding band so that the two lactone rings of dihydro-

TABLE IV
INTENSITIES OF CARBONYL ABSORPTION OF COMPOUNDS RELATED TO NOMILIN

	Lactone	$\Delta^{\alpha,\beta}$ -Lactone	Methyl Ester	Acetate	Ketone	Calc.	Found
Nomilin	2×3.9			3.2	2.2	13.2	13.1
Obacunone	3.9	5.4			2.2	11.5	11.3
Methyl obacunoate	3.9		3.8 ^a		2.2	9.9	9.8
Methyl hexahydronomilinate	3.9		3.1	3.2	2.2	12.4	11.8
α -Obacunol	3.9	5.4				9.3	9.0
Dihydroobacunone	2×3.9				2.2	10.0	9.6

^a α,β -Unsaturated ester: intensity estimated from data in Ref. 5.

The values in Tables II and III were then used to calculate intensities for the compounds discussed above, it being assumed that no serious interactions were affecting the contributions of the various groups. In the absence of information as to the environment of the carbonyl group, the intermediate value 2.2 was employed. The intensities calculated in this way agreed sufficiently well with the experimental values (Table III) to indicate that there were no further carbonyl groups to be detected, that is, that the two uncharacterized oxygen atoms of nomilin or obacunone formed ether links only. Again, had elimination of acetic acid from nomilin *not* introduced conjugated unsaturation, the calculated intensity for obacunone would have been 9.8, a much less satisfactory figure than that tabulated. The agreement between the calculated and observed figures for methyl obacunoate clearly opposed formulation of the hydrolysis as either the change of (I) into (II) (which requires the product to have an intensity greater than 11.3) or the opening of a lactone ring type (III) (which requires the product to have an intensity of at least 11.0): the observed intensity also indicated that an unsaturated rather than a saturated ring

obacunone now had (unresolved) absorption at 1735 cm.^{-1} , whereas a weaker, but sharp and distinctive, band at 1710 cm.^{-1} could confidently be ascribed to the carbonyl group. The spectrum of dimethyl octahydroobacunoninate, a glassy ester obtained from methyl hydrogen octahydroobacunoninate by means of diazomethane, confirmed this and other conclusions, since it showed a carbonyl band at 1711 cm.^{-1} , two resolved ester bands at 1754 and 1733 cm.^{-1} , and a marked band near 3μ which can be attributed to the hydroxyl group difficult to detect in methyl obacunoate.

Obacunone added hydrogen chloride in conditions under which nomilin was inert; again the absence of a band at 1620 cm.^{-1} and the reduced ultraviolet end-absorption showed that the lactonic double bond had been affected. With pyridine, obacunone hydrochloride readily regenerated obacunone. The results of perbenzoic acid titrations were curious, because nomilin could not be recovered in spite of the fact that no active oxygen had been destroyed. Obacunone did absorb one atom of active oxygen but failed to yield a crystalline product.

Comparisons between the infrared spectra of

below 1750 cm^{-1} and so we have retained the δ -lactonic formulation.

EXPERIMENTAL

Separation of nomilin and obacunone. The mixture of lactones obtained from citrus seed oil by means of aqueous methanol was fractionated from methylene chloride essentially as described by Emerson⁴ to remove limonin, but the procedure advocated by this author for the separation of nomilin and obacunone was less effective. These lactones were adsorbed too strongly on alumina to permit use of this material for chromatographic analysis, but silica gel appeared satisfactory. The crude mixture (5.00 g.) of nomilin and obacunone was chromatographed on a silica gel³⁷ column (1.6 \times 54 cm.) from benzene saturated with water. A yellow band travelled rapidly down the column and was removed; thereafter, obacunone was present in the eluate, the next 650 ml. of which contained the whole of this lactone (2.9 g.). After a second similar purification followed by one crystallization from methanol obacunone formed prisms, m.p. 229–230°, indicating a purity otherwise attainable only by many recrystallizations.

Anal. Calcd. for $\text{C}_{28}\text{H}_{30}\text{O}_7$: C, 68.68; H, 6.66. Found: C, 68.86; H, 6.71.

Nomilin (0.9 g., m.p. 254°) was eluted from the column by the use of 10% ethyl acetate in benzene (400 ml.), and after several recrystallizations from the same solvent the best specimens had m.p. 270° (decomp.). For analysis a specimen was dried at 100° at 0.001 mm. for 4 hr.

Anal. Calcd. for $\text{C}_{28}\text{H}_{34}\text{O}_8$: C, 65.33; H, 6.67; equiv. wt. (3 acid functions) 171.3. Found: C, 65.39; H, 6.76; equiv. wt. (back titration) 173.

Gummy material (0.9 g.) remaining on the column could be eluted with acetone or alcohol but was not examined further.

Obacunone with Brady's reagent gave an amorphous yellow precipitate after several hours at room temperature. When heated to boiling, a solution of obacunone in acetic acid containing a few drops of constant-boiling hydrogen bromide soon developed a mauve color. No color appeared when anhydrous hydrogen bromide in acetic acid or hydriodic acid were used; hydrochloric acid gave unreliable results. Negative reactions resulted with Tollen's, Fehling's, and Légal's reagents.

Nomilin gave a purple color in the hydrogen bromide reaction, and did not react with Tollen's, Fehling's, or Légal's reagent.

Conversion of nomilin into obacunone. Nomilin (0.4 g.) in boiling pyridine (15 ml.) was unchanged after 6 hr. Addition of a drop of water, pyridine hydrochloride (0.1 g.), or acetic anhydride (0.5 ml.) had no effect, but when the smallest possible volume of pyridine was employed the reaction occurred occasionally.

Nomilin (0.4 g.) in boiling γ -picoline (20 ml.) was converted after 1 hr. into obacunone (0.29 g.) which remained after removal of the solvent *in vacuo* and when purified from methanol formed prisms, m.p. 226–228°, not depressed by authentic material.

Nomilin (0.4 g.) was recovered intact from 6 hr. heating in boiling xylene (60 ml.) and then had m.p. and mixed m.p. 265° (decomp.).

Attempted condensation of obacunone with amines. Obacunone (150 mg.) was kept at 145–155° in aniline (1 ml.) for 30 min. The yellowish product was added to dilute hydrochloric acid and the precipitate, when purified from methanol, gave obacunone in prisms (100 mg.) m.p. and mixed m.p. 226°.

A similar experiment in which *o*-phenylenediamine hydro-

chloride (100 mg.) in pyridine (2 ml.) replaced aniline, and the time of reaction was 2 hr., gave a similar result.

Obacunoic acid. This acid was readily prepared by the method of Emerson⁴ and crystallized from aqueous acetone in needles (0.34 g.), m.p. 213° with but little decomposition. The acid had no ferric reaction in alcohol and did not react with diazotized *p*-nitraniline. For analysis, the substance was dried to constant weight at 110°/0.001 mm.

Anal. Calcd. for $\text{C}_{28}\text{H}_{32}\text{O}_9$: C, 63.93; H, 7.23. Found: C, 63.77; H, 7.01.

Potentiometric titration at 23° in 50% acetone showed obacunoic acid to be monobasic with pK_a 5.26. In the same conditions, the pK_a value of benzoic acid was 1.29 units greater than in water, therefore pK_a (water) for obacunoic acid was taken as 3.97.

The methyl ester was prepared according to Emerson⁴ and formed prisms, m.p. 172–173°, from methanol; α_D^{25} –95.9° (1 dm., 0.4663 g. per 10 ml. in acetone).

Anal. Calcd. for $\text{C}_{27}\text{H}_{34}\text{O}_9$: C, 66.67; H, 7.05. Found: C, 66.38; H, 7.08.

Attempted acetylation of methyl obacunoate. Methyl obacunoate (80 mg.), acetic anhydride (0.6 ml.), and pyridine (1 drop) were kept on the steam bath for 15 min. Addition of water (3 ml.) to the cold solution caused almost quantitative separation of methyl obacunoate m.p. and mixed m.p. 171°.

Methyl obacunoate (80 mg.) was kept in boiling acetic anhydride (1 ml.) containing sodium acetate (200 mg.) for 1 hr. Vacuum evaporation of acetic anhydride and dissolution of sodium acetate in water left a gum which did not yield to attempted crystallization or chromatographic separation on alumina.

Obacunone hydrochloride. Hydrogen chloride was slowly bubbled through a solution of obacunone (0.50 g.) in chloroform (5 ml.); after about 4 hr. the mixture solidified. The solvent was pumped off and replaced by fresh chloroform (5 ml.) which was then also pumped off. Crystallized from a mixture of Skellysolve B and ethyl acetate, the residue supplied obacunone hydrochloride in colorless leaflets (0.29 g.) which decomposed near 230°. This substance in 50% aqueous acetone containing dilute nitric acid gave no precipitate with silver nitrate: for analysis, it was dried at 100° at 0.01 mm. for 3 hr.

Anal. Calcd. for $\text{C}_{28}\text{H}_{31}\text{ClO}_7$: C, 63.61; H, 6.37; Cl, 7.23. Found: C, 63.90; H, 6.45; Cl, 7.47, 7.20.

In paraffin mulls, obacunone hydrochloride absorbed at 3100 (CH of olefin), 1725 (shoulder at 1740) (lactones), 1500 and 875 cm^{-1} (furan ring). There was no peak at 1620 cm^{-1} .

A solution of obacunone hydrochloride (0.20 g.) in pyridine (1.0 ml.) was kept at boiling point for 1 hr., after which the solvent was removed *in vacuo*. The residue was washed with a little cold methanol and then crystallized from the same solvent giving obacunone in prisms (0.12 g.) m.p. and mixed m.p. 229°. The methanol washings gave a positive reaction for chloride ions.

Nomilin (0.5 g.) in chloroform (10 ml.) was unaffected by hydrogen chloride during 3 hr. The recovered material had m.p. and mixed m.p. 275° (decomposition) and its infrared spectrum (mull) was identical with that of an authentic specimen.

α - and β -Obacunols. To obacunone (0.50 g.) in methanol (30 ml.) was added potassium borohydride (50 mg.) in a few drops of water. The crystalline precipitate which readily appeared (2–5 min.) was collected as soon as possible (since it began to redissolve when kept) and fractionally crystallized from ethyl acetate containing a little light petroleum (b.p. 60–80°). The earlier crops were purified from ethyl acetate giving α -obacunol in granules (0.18 g.) melting at 248° when slowly heated. This substance appeared to have a transition point at about 180°, at which temperature it melted when heated rapidly. The specimens for analysis were dried at 150°/0.01 mm. for 4 hr. The substance had $[\alpha]_D^{25}$ +84° (c, 0.3002 in acetone; 1 dm.).

(37) 200–300 Mesh; supplied by L. Light and Company, Ltd., Colnbrook, England.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 68.42; H, 7.08. Found: C, 68.25; H, 7.22.

The infrared spectrum of a solid film included peaks at 3500 (OH), 3100 (CH of olefin), 1730 and 1685 (lactonic absorption), 1625 (C=C), 1500 and 875 cm^{-1} (furan).

α -Obacunol (0.20 g.) dissolved in hot acetic anhydride (10 ml.) containing sodium acetate (0.50 g.) was kept on the steam bath for an hour. Removal of volatile material and addition of water to the residual glass gave an opaque semi-solid mass which was purified from aqueous methanol giving α -obacunyl acetate in prisms m.p. 216–217°. For analysis, a specimen was dried at 120°/10 mm. for 3 hr.

Anal. Calcd. for $C_{23}H_{34}O_8$: C, 67.46; H, 6.88. Found: C, 67.00; H, 7.02; MeO, negative.

In acid hydrolysis this acetate frothed excessively. After preliminary alkaline hydrolysis, this compound appeared to contain 13.79% acetyl, but α -obacunol under the same conditions gave an apparent acetyl value of 4.79; therefore the true value for the acetate was near 9.00 (calcd. for a monoacetate, 8.62).

A solid film of this acetate possessed absorption maxima at 3100 (CH of olefin), 1738, 1704 + 1698 (double maxima), 1631 (C=C), 1510 and 875 cm^{-1} (furan), but no hydroxyl absorption.

From the mother liquors of α -obacunol, thin prisms (0.05 g.) of β -obacunol separated and when repeatedly purified from the same solvent and then from methanol had m.p. 242–244°, depressed to 218° by α -obacunol, and $[\alpha]_D^{20} +72^\circ$ (c, 0.2211 in acetone; 1 dm.). For analysis, the substance was dried at 130°/0.01 mm. for 3 hr.

Anal. Calcd. for $C_{25}H_{34}O_8$: C, 68.42; H, 7.08. Found: C, 68.36; H, 7.25; MeO, negative.

In Nujol, β -obacunol had peaks at 3500 (OH), 3100 (CH of olefin), 1730 and 1692 (lactone rings), 1634 (C=C), 1515 and 877 cm^{-1} (furan). α - and β -Obacunols can most easily be differentiated in Nujol by the strong, single bands shown by α -obacunol at 1124, 1081, 827, and 812 cm^{-1} ; at these points β -obacunol has weaker bands at 1144 and 1116, 1068 and 1059, and 818 and 799 cm^{-1} .

Whereas α - and β -obacunols give only brownish colors in the hydrobromic acid test, the mother liquors gave an intense violet reaction developing rapidly even in the cold and therefore contained a third substance which has not yet been isolated. Acetylation of β -obacunol by the method successful with α -obacunol failed to give a characteristic product.

α -Obacunol (0.10 g.) in methanol (2.0 ml.) and pyridine (1.0 ml.) containing hydroxylamine hydrochloride (0.10 g.) was heated to boiling for 4 hr. Removal of volatile matter and crystallization of the product from methanol containing a drop of dilute hydrochloric acid supplied long thin prisms, which, when dried to constant weight at 110°/0.01 mm., melted over a range of about 210–240°.

Anal. Calcd. for $C_{26}H_{33}NO_8$: N, 2.97. Found: N, 1.28.

To α -obacunol (0.20 g.) in acetone (2 ml.), 0.5*N* sodium hydroxide (20 ml.) was added. When warmed, the precipitate rapidly dissolved and did not separate in the cold. Addition of dilute hydrochloric acid liberated a curdy solid which at first was soluble in aqueous sodium bicarbonate but gradually lost this property when kept. It could not be crystallized, and gave no crystalline fraction when attempts were made to purify it from ethyl acetate on a silicic acid column.

α -Obacunol (0.27 g.), in a mixture of toluene (25 ml.) and cyclohexanone (3.0 ml.) from which 10 ml. of distillate had been collected, was treated under reflux with aluminium *tert*-butoxide (0.30 g.) for 25 hr. after which the solvents were removed under vacuum and the yellowish residue was washed in chloroform with Rochelle salt in water. This treatment did not remove all the aluminum, therefore the chloroform was pumped off and the residue was precipitated from its solution in methanol by dilute hydrochloric acid. Traces of cyclohexanone were removed by precipitating the product from its solution in ethyl acetate with light petroleum. The product then crystallized from methanol giving

long prisms (0.18 g.) m.p. 248° not depressed by authentic α -obacunol.

Borohydride reduction of methyl obacunoate. This ester (0.70 g.) in methanol (15 ml.) was treated with potassium borohydride (50 mg.) in water (1.0 ml.) for 3 min. No precipitate appeared; the mixture was diluted with water and the product was extracted with chloroform, dried (Na_2SO_4) and on evaporation of the solvent formed a faintly yellow gum which gave the prompt, intense violet hydrobromic acid color test characteristic of the crude reduction product of obacunone. This gum resisted crystallization and attempts to purify it on silica from chloroform (which eluted nothing) or chloroform containing up to 10% ethyl acetate (which eluted all the material as one band). For analysis, which showed at least that the methyl ester group was intact, the gum was dried at 150°/10 mm. for 1 hr.

Anal. Calcd. for $C_{27}H_{36}O_8$: C, 66.39; H, 7.44; MeO, 6.35. Found: C, 65.67; H, 7.58; MeO, 6.82.

A similar reduction was carried out with obacunoic acid. The product, an amorphous solid, also gave an intense purple color with hydrobromic acid in acetic acid, but could not be purified.

Dihydro-obacunone. Obacunone (484 mg.) dissolved in acetic acid (20 ml.), was shaken in an atmosphere of hydrogen (at 23°; 754 mm.) with 10% palladium-on-carbon (0.5 g.). Reaction was slow and a plot of uptake against time showed no clear break. Hydrogenation was interrupted when the absorption reached the theoretical value for one double bond (ca. 26 ml.); removal of the catalyst and solvent left a gum which solidified in contact with methanol and after several recrystallizations from this solvent gave dihydroobacunone in small granular prisms (40 mg.), m.p. 183°, with a brownish-purple color reaction in the hydrobromic acid test. For analysis a specimen was dried at 100°/0.001 mm. for 2 hr.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 68.40; H, 7.06. Found: C, 68.68; H, 7.21.

In Nujol, this compound absorbed at 3100 (CH of olefin); 1735 with shoulder at 1750 (lactone rings), 1710 (C=O), 1500 and 875 cm^{-1} (furan).

Methyl dihydroobacunoate. Absorption of hydrogen by obacunone (484 mg.) in methanol (70 ml.) containing 10% palladium-on-charcoal (0.5 g.) was stopped at 30 ml. and the catalyst and solvent were removed leaving a gum which was dissolved in benzene and placed on a column of alumina (1 cm. \times 15 cm.). Elution with 10% ethyl acetate in benzene removed no significant material, but ethyl acetate eluted a gum which crystallized from methanol in prisms (30 mg.), m.p. 145°. This substance, methyl dihydroobacunoate, was also obtained from the crude hydrogenation product by repeated extraction with Skellysolve B containing a little benzene followed by concentration of the extracts and crystallization of the residue from methanol. It gave a bluish-purple color in the hydrobromic acid test and had an ultraviolet spectrum almost identical with that of dihydroobacunone. The analytical specimen was dried to constant weight at 100° at 0.001 mm.

Anal. Calcd. for $C_{27}H_{36}O_8$: C, 66.38; H, 7.43; MeO, 6.35. Found: C, 66.68; H, 7.62; MeO, 6.12.

In Nujol, this ester had absorption bands at 3500 (OH), 1730 and 1712 (lactones and C=O), 1500 and 875 cm^{-1} (furan).

Methyl hydrogen octahydroobacunoninate. (a) Obacunone (0.5 g.) in acetic acid (15 ml.) containing 1% palladium-on-alumina (0.5 g.) was shaken under hydrogen until the maximum absorption (113 ml.) was observed (about 6 hrs.). The residue from the filtered and evaporated solution was partly soluble in aqueous sodium hydrogen carbonate. Regained by acidification of the solution with 2*N* sulphuric acid, methyl hydrogen octahydroobacunoninate crystallized from methanol in sheaves of needles (130 mg.) which melted at 175–177° with evolution of gas but no charring. For analysis, a specimen was dried at 120°/15 mm. for 3 hr.

Anal. Calcd. for $C_{27}H_{44}O_8$: C, 65.30; H, 8.93; MeO, 6.25;

equiv. wt. (1 carboxyl), 496. Found: C, 65.15; H, 8.62; MeO, 6.23; equiv. wt. (titration with 0.01N NaOH), 464.

(b) In the presence of 1% palladium-on-alumina (1 g.), methyl obacunoate (0.50 g.) in acetic acid (15 ml.) absorbed 116 ml. of hydrogen (calcd. for 5 double bonds, 125 ml.) in 2 hr. The gum left after filtration and evaporation of the solvent was almost entirely soluble in sodium bicarbonate solutions and when purified from methanol had m.p. 175–177° (with gas evolution). Losses during recrystallization were considerable; the yield of pure material was approximately 290 mg. at best. The substance was identified as methyl hydrogen octahydroobacunoninate by infrared spectral comparison of dried material. It did not react easily with 2,4-dinitrophenylhydrazine sulfate and gave a weak red color in the hydrobromic acid test.

Potentiometric titration in 50% aqueous acetone showed one inflection only and gave pK_a 4.13. In the same circumstances, benzoic acid suffered a solvent shift of 1.24 units. Methyl hydrogen octahydroobacunoninic acid in water therefore has pK_a 2.89.

Dimethyl octahydroobacunoninate was obtained as a clear glass by interaction of the foregoing acid in ether with diazomethane. When a solution in ether was allowed to evaporate slowly, prisms losing solvent and melting near 80° resulted, and as no conventional crystallization procedure was successful, this operation was repeated several times to purify the substance. Further purification was effected from benzene on a silica column, when a little gum was removed. The main fraction, which appeared when 10% ethyl acetate in benzene was the eluent, was still uncrystallizable, and was therefore dried at 150°/15 mm. for 2 hr. for analysis. In acetone it had $[\alpha]_D^{24.5} +128$ (c, 0.07336; 1/2 dm. tube).

Anal. Calcd. for $C_{28}H_{46}O_8$: C, 65.86; H, 9.08; MeO, 12.16. Found: C, 66.21; H, 9.30; MeO, 12.57.

The infrared spectrum of this ester had absorption maxima at 3500 (OH), 1754 and 1733 (lactones), and 1711 cm^{-1} (C=O). There were no peaks at 3100, 1500, or 875 cm^{-1} .

Hexahydronomilinic acid. Shaken under hydrogen with 5% palladium-on-charcoal (1.0 g.), nomilin (0.50 g.) in acetic acid (15 ml.) absorbed 103 ml. in 3 hr. (theory for 4 double bonds, 105 ml.). Isolated in the usual way, hexahydronomilinic acid separated from methanol in thin rectangular plates (0.36 g.) melting at 223° with evolution of gas (carbon dioxide was indicated by a positive test with baryta) but without charring. This acid was readily soluble in aqueous sodium hydrogen carbonate, gave no color in the hydrobromic acid test, and no color with tetranitromethane. It retained methanol of crystallization strongly. For analysis a sample was dried at 110°/0.001 mm. for 3 hr.

Anal. Calcd. for $C_{28}H_{42}O_8 \cdot MeOH$: C, 62.80; H, 8.36; MeO, 5.60; equiv. wt. (one carboxyl) 554. Found: C, 63.00; H, 8.67; MeO, 5.69; equiv. wt. 546 (titration with 0.01N NaOH).

After being dried at 150°/0.01 mm. for 10 hr. this acid gave a negative result in the Zeissel determination but still had m.p. 223° and when recrystallized from methanol took up this solvent once more giving material indistinguishable from the analytical specimen.

In potentiometric titration in 50% acetone, hexahydronomilinic acid behaved as a monobasic acid pK_a 3.92. In the same conditions, the solvent correction for benzoic acid from 50% acetone to pure water was -1.30, therefore hexahydronomilinic acid had pK_a (water) 2.62.

A solution of hexahydronomilinic acid (327 mg.) in aque-

ous sodium hydrogen carbonate (10 ml.) was kept at 0°. Ten minutes after the addition of 2.8% hydrogen peroxide (1.5 ml.) the solution was acidified with 2N sulfuric acid and the precipitate, when crystallized from methanol, gave prisms (280 mg.) m.p. 205° (decomposition). A second crystallization gave pure hexahydronomilinic acid identified by its infrared spectrum and melting characteristics.

Methyl hexahydronomilinate. The rapid reaction between hexahydronomilinic acid in acetone and diazomethane in ether gave methyl hexahydronomilinate; isolated by evaporation of the solvents and purified from methanol, this ester formed plates melting at 222° without decomposition and having in acetone $[\alpha]_D^{23} +74^\circ$ (c, 1.1868, 1/2 dm. tube). For analysis, a sample was dried at 110°/0.01 mm. for 3 hr.

Anal. Calcd. for $C_{29}H_{44}O_9$: C, 64.92; H, 8.29. Found: C, 65.14; H, 8.11.

As a solid film, this compound had peaks at 1725 with shoulder at 1740 (lactone rings and acetate), and 1700 cm^{-1} (ill-resolved) (C=O). There were no bands at 3500, 3100, 1500, or 875 cm^{-1} .

Ozonolysis of obacunone. Ozonized oxygen was passed into a solution of obacunone (2.0 g.) in ethyl acetate (100 ml.) at 20°. After 45 min. obacunone could no longer be detected in the solution and the solvent was removed *in vacuo* leaving a yellowish glass which was powdered under water and left for 12 hr. The mixture gave no reaction with the starch-iodide reagent. A current of air passed through the solution did not affect aqueous 2,4-dinitrophenylhydrazine sulphate but with baryta gave a precipitate which effervesced with dilute acids. The solid ozonolysis product was warmed with methanol but still failed to crystallize: it gave a red ferric reaction, reduced Fehling's solution slowly, and gave an orange (amorphous) precipitate with 2,4-dinitrophenylhydrazine sulphate in aqueous alcohol but no color with hydrogen bromide.

Purification of the crude product dissolved in chloroform was effected with a silica column (1 × 20 cm.). Fourteen fractions (each 200 ml.) were collected, but only the third, fourth, and fifth gave partially crystalline material. This formed colorless prisms m.p. 274° (decomp.) when crystallized from a mixture of methanol and acetone and then methanol. The pure material did not affect Fehling's solution or ferric chloride and was insoluble in cold 2N sodium hydroxide.

Anal. Calcd. for $C_{26}H_{32}O_8$: C, 66.09; H, 6.83; MeO, 6.57; equiv. wt. (3 carboxyls) 157.2. Found: C, 66.18, 66.19; H, 6.75, 6.88; MeO, 6.87, 6.79; equiv. wt. (back titration), 157.

In ethanol, this compound has λ_{max} 244 $m\mu$ (ϵ , 16.1×10^3) with a shoulder at 220 $m\mu$ (ϵ , 10.4×10^3).

Spectra. The majority of the infrared spectra were determined by means of a Perkin-Elmer Spectrophotometer 21B. Intensity measurements were carried out in chloroform solution as in Method III of ref. 28 and with the approximations for polycarbonyl compounds used there. Ultraviolet spectra were determined in ethanol using a Cary recording spectrophotometer.

Analyses. Analyses were determined by Heather King of the University of California, Los Angeles, and by Adam Inglis of the University of Liverpool.

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