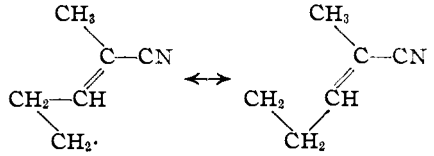
This is a clear indication that the increase in rate when R = isobutyl is not due to "B" strain[13] since models indicate that "B" strain should be increased considerably on going from R = isobutyl to R = *t*-butyl. From Table I it can be seen that the decomposition of the azo compound derived from R = *t*-butyl is slightly slower than that derived from R = methyl[2a] at 80.2°. Both of these rates are much smaller than that of the azo compound when R = isobutyl.

The azo compound derived from methyl cyclopropyl ketone decomposes much more rapidly than either of the two other azo compounds described in this paper or any previously reported.[2a] A steric explanation of the type discussed above is not adequate or satisfactory. Semi-accurate models (it is impossible to construct a cyclopropane ring with Fisher–Hirschfelder models) indicate some crowding of the groups around each carbon due to the rigidity of the cyclopropyl group. This crowding is not present when R = isopropyl, because of the fact that the methyl groups can easily fold back. Thus, there may be an increased tendency for the cyclopropyl group to release a slight "B" strain[13] by forming the planar radical. A second explanation may be due to the increased possibilities for hyperconjugation in a cyclopropyl group contributing to increased resonance in the radical formed on decomposition. Thus, the following resonance forms can be written, in addition to those involving resonance through the nitrile group:



Klotz[14] in a study of the ultraviolet absorption spectra of compounds containing the cyclopropyl group conjugated with an olefinic or carbonyl double bond found that these compounds absorb light at longer wave lengths than the corresponding non-cyclic compounds. Similar results have been obtained by Rogers with cyclopropylbenzene and by Mariella, Peterson and Ferris[15] with 2-cyclopropylpyridine. This shift in absorption has been attributed to hyperconjugation of the three-membered ring with the double bond involving ionic resonance forms.

A discussion of errors arising from this type of measurement was included in reference 2a. For the most part, induction periods have been eliminated by careful exclusion of oxygen by nitrogen. In addition, temperature control has been improved.

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[13] H. C. Brown, H. Bartholomay and M. D. Taylor, THIS JOURNAL, **66**, 435 (1944).

[14] I. M. Klotz, *ibid.*, **66**, 88 (1944).

[15] M. T. Rogers, *ibid.*, **69**, 2544 (1947); R. P. Mariella, L. F. S. Peterson and R. C. Ferris, *ibid.*, **70**, 1474 (1948); see also J. D. Roberts, W. Bennett and R. Armstrong, *ibid.*, **72**, 3329 (1950), who have suggested that the abnormally high rate of solvolysis of cyclopropol carbinyl chloride is due to stabilization of the cyclopropyl carbinyl cation by hyperconjugation involving the cyclopropane ring.

BROOKLYN, N. Y. RECEIVED NOVEMBER 14, 1950

[CONTRIBUTION FROM THE ENZYME RESEARCH DIVISION, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, UNITED STATES DEPARTMENT OF AGRICULTURE]

Bitter Principles of Citrus. II. Relation of Nomilin and Obacunone[1]

BY OLIVER H. EMERSON

Obacunone was isolated from Florida citrus seed oil, along with limonin and nomilin. Nomilic acid, previously prepared from nomilin, is identical with obacunoic acid. Nomilin is probably an acetoxy-dihydroöbacunone, with the acetoxy group in *β*-position to the carbonyl of one of the lactone groups.

Limonin, $C_{26}H_{30}O_8$, has been shown to be the bitter principle that develops on standing in the expressed juice of navel oranges.[2a,b] A similar compound, nomilin, $C_{28}H_{34}O_9$, likewise of bitter taste, occurs with limonin in the seeds of oranges and lemons. Since citrus seeds are among the best practical sources of limonin, it was hoped that the oil expressed from mixed citrus seeds, which is a commercial product of Florida, would prove an advantageous source for the preparation of large amounts of limonin. Although the bitter substances could be readily extracted from the oil, the yield of limonin was disappointing, as the chief bitter material proved to be nomilin. A third beautifully crystalline substance whose analysis indicated it to have the composition $C_{26}H_{30}O_7$ was also obtained. Although not bitter, its close relationship to nomilin was shown by the fact that hydrolysis with dilute aqueous alkali converts both substances to the same $C_{26}H_{32}O_8$ acid. The data in Table I show that this third substance is identical with obacunone isolated by Kaku and Ri[3] from the bark of *Phellodendron amurense*, and very probably also with casimirolid, obtained by Power and Callan[4] from the seeds of *Casimiroa edulis*. It is of interest that Phellodendron and Casimiroa belong to the same family (*Rutaceae*) as does Citrus, and that Kaku and Ri[3] found obacunone to occur with limonin.

The slight difference assigned by Power and Callan[4] to the composition of their product could easily be explained by difficulties in combustion, since many complex natural products are notoriously hard to burn in macro-determinations. The English workers prepared their methyl ester by treating the acid with methanolic hydrogen chloride, but the precise conditions were not stated

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[1] Enzyme Research Division Contribution No. 132. Article not copyrighted.

[2] (a) O. H. Emerson, THIS JOURNAL, **70**, 545 (1948); (b) O. H. Emerson, *Food Technology*, **3**, 248 (1949).

[3] T. Kaku and H. Ri, *J. Pharm. Soc. Japan*, **55**, 222 (1935), *C. A.*, **31**, 6643 (1937).

[4] F. B. Power and T. Callan, *J. Chem. Soc.*, **99**, 2004 (1911).

TABLE I

	Substance from citrus seed oil	Obacunone	Casimirolid
Assigned formula	$C_{26}H_{30}O_7$	$C_{26}H_{30}O_7$	$C_{24}H_{28}O_6$
Melting point, °C.	229–230	229–230	229–230
$[\alpha]_D$	−50.2°	−50.9°	−49.2°
Acid, formula	$C_{26}H_{32}O_8$	$C_{26}H_{32}O_8$	$C_{24}H_{30}O_7$
Acid, m.p., °C.	208–209	208–209	207
Acid, $[\alpha]_D$	−89.6°[a]	−87.4°	−86.8°[a]
Acid, methyl ester m.p., °C.	177–178	172–173 108–110	108–110
Acid, acetyl derivative m.p., °C.	145	154–155	142

[a] In ethanol.

Kaku and Ri[3] obtained two methyl esters, while in this Laboratory, using diazomethane, only one was obtained in excellent yield. In view of the sensitivity of substances of the limonin type to strong mineral acids,[5] it is possible that the esterification procedure of Power and Callan[4] resulted in other changes in the molecule.

However, in view of the possible question of the identity of our third substance with casimirolid, we will adopt the name obacunone, and the name obacunoic acid for its hydrolysis product. The name nomilic acid[2a] will for reasons of priority be dropped.

The name obacunone implies that the substance is a ketone, although no carbonyl derivatives had previously been described. Fortunately for the name, the substance readily forms a beautifully crystalline oxime by the oximation procedure of Bachmann and Boatner.[6] Hence, obacunone is, like limonin and nomilin, a keto-dilactone. Like these substances and obacunoic acid, obacunone gives a strong red color with concentrated sulfuric acid, and a rather weak yellow color with tetranitromethane. In contrast to the other substances, obacunone readily reduces an acetone solution of potassium permanganate.

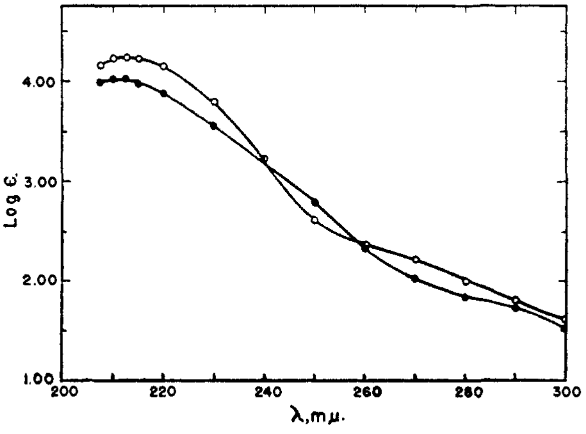

Fig. 1.—Absorption spectra: O—O, obacunone in methanol; ●—●, obacunoic acid in ethanol. Although the region below 220 mμ is beyond the most accurate range of the instrument, the curves are certainly at least qualitatively correct.

Kaku and Ri[3] considered obacunone to contain one double bond because of the formation of a dibromide decomposing at 175–176°. However, on treating a chloroform solution of obacunone with bromine, we observed the formation of much hydrogen bromide and were unable to isolate any definite compound.

In addition to the ready cleavage of nomilin to acetic acid and obacunoic acid with dilute aqueous alkali, refluxing nomilin with acetic anhydride and pyridine converts that substance directly to obacunone. This suggests that nomilin is an acetoxydihydro obacunone with the acetoxy group in β-position to one of the lactone groups. The strong absorption of obacunone and obacunoic acid in the further ultraviolet with maxima between 210 and 215 mμ (Fig. 1) is in keeping with an α,β-unsaturated lactone, or, perhaps in the case of obacunoic acid, an α,β-unsaturated acid.

Experimental

Isolation of the Compounds.—Commercial raw citrus seed oil, expressed early in the 1950 season by the Hutchman Company of Lakeland, Florida, was mixed in batches of 5 l. with 10 l. of petroleum ether, b.p. 88–98° (Skellysolve C) and filtered through super-cel.[7] Some bitter material separated, which was extracted with acetone and added to the main bitter fraction. The petroleum ether solution was then extracted with 95% methanol; three extractions with 2 l. each time removed the bitter material practically completely. The extraction was conducted so that methanol extracts of partly debittered oil were used to extract fresher batches. In all, 30 gallons of citrus seed oil was used. The crude bitter fraction obtained by concentrating the methanol and acetone extracts was worked up as previously described.[2a] In all, 20 g. of limonin and 80 g. of nomilin were obtained.

Obacunone was obtained from the mother liquors of limonin and nomilin. It was separated from the former by its much greater solubility in isopropyl alcohol and from the latter by its much greater solubility in hot toluene. Further purification was secured by recrystallization from acetone, and then from ethyl acetate. The best preparation melted at 229–230°, although this melting point was not easy to attain. The solubilities of obacunone in general parallel those of limonin and nomilin, but as might be expected from the much lower melting point, they are in general very considerably greater. About 30 g. of obacunone was obtained, although the amount present was undoubtedly very much greater.

For analysis, one sample was recrystallized from toluene and one from acetone, and both were dried to constant weight at 100° at 0.1 mm. The preparation from toluene crystallized solvent-free, while that from acetone contained 1 molecule of acetone of crystallization; loss on drying, 10.95%; calcd. for $C_{26}H_{30}O_7 + C_3H_6O$: 11.3%.

Anal. Calcd. for $C_{26}H_{30}O_7$: C, 68.71; H, 6.64. Found: preparation from toluene, C, 68.64; H, 6.74. Preparation from acetone, C, 68.77; H, 6.63; $[\alpha]^{26}_D$ −50.2° (*c*, 2.14, chloroform).

Obacunoic Acid.—Obacunone (233 mg.) was suspended in 25 ml. of 0.1 *N* aqueous sodium hydroxide and heated on the steam-bath with swirling and grinding of the larger crystals until solution was almost complete (40 minutes). Small amounts of acetone were added to hasten the process. The solution was filtered, cooled and acidified. The crude acid was filtered and recrystallized from a small amount of dilute acetone, yielding a product melting at 206–208°, 202 mg. (84%). A second recrystallization brought the melting point up to 208–209°. On admixture with the $C_{26}H_{32}O_8$ acid of the same melting point previously obtained from nomilin,[2a] there was no melting point depression. Dr. Francis T. Jones of the Western Regional Research Laboratory, Albany, California, kindly examined the crystallographic properties of the two preparations, and found them

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[5] T. A. Geissman and V. Tulagin, *J. Org. Chem.*, **11**, 760 (1946).

[6] W. E. Bachmann and C. H. Boatner, THIS JOURNAL, **58**, 2097 (1936).

[7] Mention of these products by name does not constitute recommendation over similar products not named.

identical.[8] Rotation: $[\alpha]^{25}\mathrm{D}$ −89.6° (*c*, 1.115, absolute ethanol), $[\alpha]^{26}\mathrm{D}$ −97.0° (*c*, 1.25, acetone).

Methyl Obacunoate.—Obacunoic acid (242 mg.) dissolved in a few ml. of acetone was treated with a slight excess of an ether solution of diazomethane. After standing a few minutes, the solvents were pumped off, and the residue taken up in a small amount of *t*-butyl alcohol. Crystallization was obtained by adding a little petroleum ether, yield 222 mg., m.p. 175–177°. A second recrystallization brought the melting point to 177–178°. For analysis the substance was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{27}H_{34}O_8$: C, 66.65; H, 7.05. Found: C, 66.54; H, 7.02.

Acetyl Obacunoic Acid.—Obacunoic acid (100 mg.) was acetylated by heating on the steam-bath for one hour with 2 ml. of acetic anhydride and 50 mg. of fused, powdered sodium acetate. After pumping off the excess acetic anhydride, the residue was washed with water and crystallized from a mixture of ethyl acetate, benzene and isoöctane, m.p. about 145°, although not very sharp. For analysis the product was dried as described above.

Anal. Calcd. for $C_{28}H_{34}O_9$: C, 65.36; H, 6.65. Found: C, 65.84; H, 6.95.

Obacunone Oxime.—Obacunone (112 mg.) with an equal weight of hydroxylamine hydrochloride was refluxed four hours with 2 ml. of absolute ethanol and 2 ml. of pyridine. The solvents were pumped off, and the residue washed with water, and crystallized from a small amount of methanol, in which it was quite soluble. The substance crystallized in fine prisms which decomposed when heated above 200°, rapidly above 230° yielding a dark red oil. For analysis the substance was dried to constant weight at 100° and 0.1 mm.

Anal. Calcd. for $C_{26}H_{31}NO_7$: C, 66.52; H, 6.65; N, 2.98. Found: C, 66.85; H, 6.97; N, 3.01.[9]

Conversion of Nomilin to Obacunone.—Nomilin (520 mg.) was refluxed for two hours with a mixture of 5 ml. of acetic anhydride and 5 ml. of pyridine. After the solvents were pumped off, the residue was crystallized from acetone, yielding 289 mg. of a slightly impure obacunone. A second recrystallization brought the melting point up to 227–229°, and the product gave no depression on admixture with natural obacunone. Dr. F. T. Jones, of the Western Regional Research Laboratory, examined the optical properties of the crystals, and found they checked perfectly with those of natural obacunone from the citrus seed oil.

Acknowledgment.—The author wishes to express his sincerest thanks to Dr. C. W. Koch and Mr. V. H. Tashinian of the Chemistry Department of the University of California, Berkeley, California, for the microcarbon–hydrogen determinations, and to the following gentlemen of the Western Regional Research Laboratory, Albany, California: Messrs. Glen F. Bailey and Stanley Friedlander for the measurements of the absorption spectra, Dr. R. M. McCready for the polarimetric measurements and Dr. F. T. Jones for the microscopic examination of many preparations.

ALBANY, CALIF. RECEIVED NOVEMBER 20, 1950

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[8] A description of the crystallographic properties of the substances described in this paper is to appear shortly.

[9] Dumas analysis by Walter Mann, Jr.

[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

The Role of Phenol in the Reaction of 4,7-Dichloroquinoline with Novol Diamine

BY ALEXANDER R. SURREY AND ROYAL A. CUTLER

A study was undertaken to investigate the role of phenol in the reaction of 4-chloroquinolines with aliphatic amines. Evidence is presented for a proposed mechanism involving the formation of 4-phenoxyquinoline hydrochloride as an intermediate. The effect of acid and base on the reaction is also reported.

It is generally known that in the 9-chloroacridine series the use of phenol[1] greatly facilitates the reaction with aliphatic amines. It is not surprising, therefore, to find a carry-over of the use of phenol into the quinoline series. However, although there are numerous examples in which phenol has been used in the condensation of aliphatic amines with 4-chloroquinolines, only isolated statements have been reported regarding its efficacy in this series. For example, Walker[2] stated that "occasionally phenol has been added as a catalyst but it does not appear to have the same marked influence in the quinoline series as it has with 9-chloroacridines." Kenyon, Wiesner and Kwartler[3] stated that 4,7-dichloroquinoline reacts with molten phenol (by analogy with the 9-chloroacridine series) to form the phenoxy compound which then reacts with novol diamine at 135° to give chloroquine. Actually, there has been no conclusive evidence that the phenoxy quinoline (hydrochloride) is the intermediate in these reactions. One objection to the formation of the latter as an intermediate is that it involves the reaction of phenol in preference to the more strongly nucleophilic amine. On the other hand, one might picture the phenol as exerting primarily a solvolytic effect on the 4-chlorine thus making the 4-carbon more susceptible to attack by the amine.[4]

In connection with some of our work on the reaction of 2- and 4-chloroquinolines with secondary amines[5] and particularly in the preparation of chloroquine from the condensation of 4,7-dichloroquinoline with novol diamine,[6] N^1,N^1-diethyl-1,4-pentanediamine, it seemed desirable to investigate more carefully the role of phenol in these reactions.

The beneficial effect of phenol can be demonstrated very clearly in the reaction of some 2- and 4-chloroquinolines with secondary amines.[5] In the presence of phenol the reactions occurred readily and the products were obtained in good

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[1] (a) The use of phenol as a reaction medium was introduced by O. J. Magidson and A. M. Grigorowski, *Ber.*, **66**, 866 (1933). (b) In a later paper, *ibid.*, **69**, 346 (1936), these authors showed that the 9-chloroacridines can react with phenol to form the 9-phenoxyacridines, and that the latter can react with an amine to form the 9-amino derivative. On this basis, it was postulated that the 9-phenoxyacridine was an intermediate in the reaction of a 9-chloroacridine with an amine in the presence of phenol.

[2] J. Walker, *J. Chem. Soc.*, 1552 (1947).

[3] R. L. Kenyon, J. A. Wiesner and C. E. Kwartler, *Ind. Eng. Chem.*, **41**, 654 (1949).

[4] The strong solvolytic action of phenol toward halogen has been demonstrated; (a) P. D. Bartlett and H. J. Dauben, THIS JOURNAL, **62**, 1339 (1940). Its marked effect in promoting ether formation in the case of triphenylmethyl halides is particularly demonstrative of this point; (b) C. G. Swain, *ibid.*, **70**, 1119 (1948).

[5] Work to be published.

[6] A. R. Surrey and H. F. Hammer, THIS JOURNAL, **68**, 113 (1946).