close to those finally arrived at as approximately correct for the pure *cis*-isomer. The inversion proceeded rapidly and was practically complete in 24 min.

For preparation at higher concentrations, 25 g. of the *trans*-isomer in 2 l. of *n*-pentane required exposure to bright sunlight for 10 days. The course of the isomerization was followed by spectroscopic examination of portions of the product at intervals of 6, 10 and 11 days; the $\epsilon_{252.5\ m\mu}$ were 18.2, 18.8 and 18.8 × 10^3, respectively, and the $\epsilon_{297.5\ m\mu}$ were 9.45, 9.1 and 9.1 × 10^3. The *cis*-isomer (V), isolated by evaporation of the solvent, was a yellow oil which could not be induced to crystallize from a variety of solvents at temperatures ranging down to −60°. It could not be safely distilled because of slow isomerization to the *trans*-isomer at the temperature involved.

The best sample of the oil, of $\epsilon_{252.5\ m\mu}$ 18.8 × 10^3, was shown to be nearly pure by analysis, molecular weight, ultraviolet absorption characteristics, subsequent isomerizations to the *trans*-isomer, reactions parallel with those of the *trans*-isomer, including conversion into α-bromo-β-morpholinylbenzylacetophenone[7] in good yield, and by practically complete solubility in a saturated solution of the *trans*-isomer in 40% aqueous methanol at 25.0°. The solubility of the *trans*-isomer (37.7 mg. per 25.0 ml.) was determined by evaporating a measured volume under a stream of nitrogen and drying *in vacuo* at 0.5 mm. pressure (the reliability of this measurement was shown by resubjection of the residue to the evaporation procedure for 10 hr. and the loss of only 1.0 mg. in that time). The complete solubility of a sizable amount of the *cis*-isomer in this solution was shown by dissolving 52.3 mg. in 50 ml. of the saturated *trans* solution at 25.0°, thermostating in the presence of added 10 mg. of the *trans*-isomer as a seed, and evaporation of a 25.0-ml. aliquot of the solution; residue 64.2 mg. (this is exactly the amount calcd. on the basis of complete solubility of the added *cis*-isomer). This result shows that the product of irradiation contained practically none of the *trans*-isomer, but it must be emphasized that the possibility of the presence of small amounts of other impurities has not been excluded. There is evident here an almost quantitative conversion at photoequilibrium to the *cis*-isomer, and little if any resinification or formation of other by-products, and the physical constants determined appear to be close to correct: $n^{24.4}{}_D$ 1.6334; $d^{24.4}$ 1.3691; M_D 74.95 (calcd. 71.58, exaltation 3.37).

Anal. Calcd. for $C_{15}H_{11}BrO$: mol. wt., 387.2; C, 62.74; H, 3.86. Found: mol. wt., 286.2; C, 62.99; H, 4.33.

It was evident from consistently high analyses for hydrogen that an appreciable though small amount of impurity was present. This impurity has been shown to be a hydrocarbon residue (2.2 mg. per 100 ml.) left upon evaporation of the *n*-pentane used, and also formed upon irradiation of the solvent for 10 days (4.4 mg. per 100 ml.). (Unfortunately this exposure was not nearly as effective as in the experiment above, because of weather.) On this basis the analytical sample contained at least 2.6% of impurity of composition presumably $(CH_2)_n$, and the above analysis corrected for the contribution of this impurity is C, 62.78; H, 4.13, with the hydrogen value within the acceptable range. This amount of impurity would have had a small but appreciable effect on the physical constants and would involve a correction on the order of $n^{24.4}{}_D$ +0.0055; $d^{24.4}$ +0.0054; exaltation M_D +0.21; $\epsilon \times 10^3$ at λ_{max} 252.5 mμ, +0.49, and at λ_{max} 297.5, +0.24.

Inversion of *cis*-α-Bromobenzalacetophenone.—Solutions of 2.5 g. of the *cis*-isomer (V) in 25 ml. of chloroform, one containing a quantity of iodine sufficient to lend a persistent and distinct color, were exposed to bright sunlight for 90 min. The products were recovered by evaporating the solvent, dissolving in 10 ml. of ethanol and refrigerating at −20° for 24 hr. Only the product from the iodine-catalyzed reaction crystallized; yield 1.0 g., m.p. 40–42°; second crop, 0.8 g., m.p. 39–42° (total yield 72%, identified as *trans*-isomer by mixture m.p.). The residue from the portion irradiated in the absence of iodine could not be induced to crystallize despite seeding with the *trans*-isomer, and here, as in the case of the *n*-pentane solution, the photoequilibrium evidently lies well over on the side of the *cis*-isomer.

The *cis*-isomer was isomerized also (a) by heating at 160° for one hour under an atmosphere of nitrogen, and (b) by catalytic action of iodine and heating at 100° under nitrogen; the yields of *trans*-isomer of correct m.p. were 67 and 60%, respectively.

The *cis*-isomer was recovered unchanged after treatment with 0.5 equivalent of potassium acetate in ethanol under refluxing for 2 and for 9 hours; the recovered oils which resisted efforts to crystallize them, were identified by extinction coefficients at the two λ_{max}.

CHARLOTTESVILLE, VA. RECEIVED APRIL 16, 1951

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

The Structure of Humulone and Lupulone as Revealed by Ozonization[1a]

BY J. F. CARSON

This paper reports the ozonization of the two important constituents of hops, humulone and lupulone, the results of which require a modification in the currently accepted structures of these compounds. The ozonization of humulone I and of lupulone III yielded acetone isolated as the 2,4-dinitrophenylhydrazone in yields, respectively, of 1.26 and 2.09 moles per mole of compound. In neither case could isobutyraldehyde be detected. These results prove that the side chain attached to C_4 of humulone and the two side chains attached to C_4 of lupulone should be represented as $(CH_3)_2C{=}CH{-}CH_2{-}$. The data confirm the accepted structure of the side chain attached to C_6 of humulone and lupulone.

The structure of the side chain attached to C_4 of humulone I was considered by Wieland[2] to be $(CH_3)_2CH{-}CH{=}CH{-}$ because in the alkaline degradation of humulone I to humulinic acid II, isobutyraldehyde and isohex-2-enoic acid were isolated. The position of the double bond in the side chain attached to C_5 in humulinic acid was established by Wieland and Martz.[3] Ozonization of humulinic acid yielded acetone, which revealed the position of this double bond and also, assuming no rearrangement, the position of the double bond in the chain attached to the C_6 of humulone I. In the case of lupulone III, no similar alkaline degradation was possible and the two side chains attached to C_4 were assumed by Wöllmer[4] to be $(CH_3)_2CH{-}CH{=}CH_2{-}$ by analogy with humulone I. Similarly the position of the double bond in the side chain on C_6 of lupulone was assumed as shown in III without proof.

Recently, Cook and Harris[5] have reported that in their hands, the alkaline degradation of humulone yielded isohex-3-enoic acid instead of the

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(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

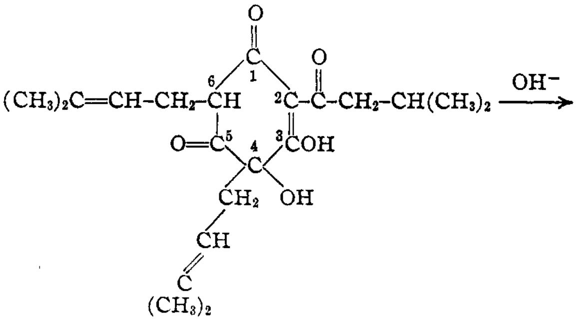
(1a) NOTE ADDED IN PROOF.—Full priority for the revised structure of lupulone is due to the work of M. Verzele and F. Govaert (*Bull. soc. chim. Belg.*, **58**, 432 (1949)) which escaped our notice. These investigators, on the basis of alkaline degradation and ozonization, have arrived at the same structure for lupulone as advanced here.

(2) H. Wieland, *Ber.*, **58**, 102 (1925).

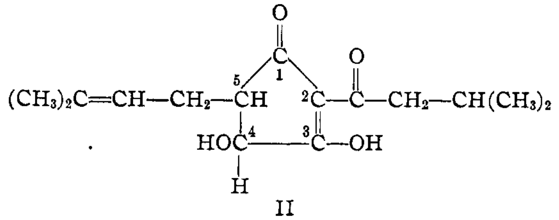
(3) H. Wieland and E. Martz, *ibid.*, **59**, 2352 (1926).

(4) W. Wöllmer, *ibid.*, **58**, 672 (1925).

(5) A. H. Cook and G. Harris, *J. Chem. Soc.*, 1873 (1950).

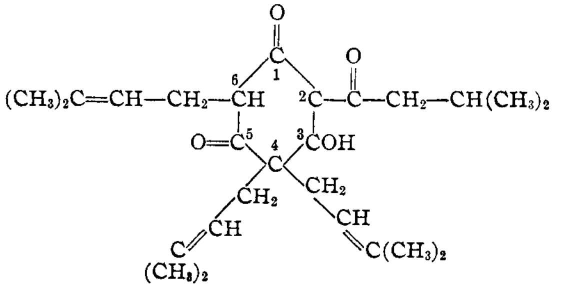


I



II

+ $(CH_3)_2CHCHO + CH_3COOH + (CH_3)_2CHCH{=}CHCOOH$



III

isohex-2-enoic acid reported by Wieland[2] and that both acetone and isobutyraldehyde were formed. We have confirmed this observation, in part, to the extent that in several preparations of humulinic acid, acetone has been identified by chromatography of the 2,4-dinitrophenylhydrazones.[6] These observations are not surprising, since the double bond may migrate in alkali to give an equilibrium mixture, $(CH_3)_2C{=}CH{-}CH_2{-} \rightleftarrows (CH_3)_2CH{-}CH{=}CH{-}$ and both acetone and isobutyraldehyde and two unsaturated acids might be expected. For the same reason, the location of the double bond in humulinic acid II may not necessarily reflect the position of the double bond in humulone. I. Alkaline degradation of humulone is, therefore, of doubtful value in establishing the positions of the double bonds and, to this extent, the actual structures of humulone and lupulone have been uncertain.

To establish the positions of the double bonds in these compounds, the ozonization of humulone, lupulone and humulinic acid has been investigated. The volatile carbonyl components resulting from decomposition of the ozonides were removed by distillation and converted to the 2,4-dinitrophenylhydrazones which were chromatographed on silicic acid. By this method, mixtures of the dinitrophenylhydrazones of formaldehyde, acetaldehyde, acetone and isobutyraldehyde are easily separated. If the formulas for humulone and lupulone as proposed by Wieland[2] and Wöllmer[4] are correct, both compounds should yield acetone and isobutyraldehyde. If the proposed structures I and III are correct, acetone, but no isobutyraldehyde, should result from ozonization.

Ozonization of humulinic acid in acetic acid for one hour yielded acetone isolated as the 2,4-dinitrophenylhydrazone in a 61 molar % yield[7] confirming the results of Wieland[3] in agreement with structure II. Ozonization of humulone under the same conditions yielded acetone, 126 mole % based on one isopropylidene group or 63 mole % based on two of these groups. No isobutyraldehyde was formed. Lupulone, on ozonization for one hour, yielded acetone in a 198% yield based on one isopropylidene group or 66% based on three such groups. When lupulone was ozonized for two hours, the yield of acetone dinitrophenylhydrazone was 209% based on one isopropylidene group or 70% based on three such groups. When ozonization of humulone and lupulone was extended for six hours, complex mixtures were obtained as shown by chromatography. No crystalline acetone dinitrophenylhydrazone could be isolated. Small quantities of the dinitrophenylhydrazone of methyl *n*-propyl ketone were obtained, 1.5 mole % from humulone and 12 mole % from lupulone. The formation of this compound probably results from a rearrangement during ozonization or during decomposition of the ozonide. These results point out the necessity of investigating conditions of ozonization in structural determinations.[8]

The isolation of acetone from the ozonization of humulone in a yield of 1.26 moles per mole of humulone and the absence of isobutyraldehyde proves that two of the side chains of humulone have the structure $(CH_3)_2C{=}CH{-}CH_2{-}$ as shown in formula I. Similarly the isolation of acetone from the ozonization of lupulone in a yield of 2.09 moles/mole of lupulone and the absence of isobutyraldehyde proves that three of the side chains have the structure $(CH_3)_2C{=}CH{-}CH_2$ as shown in formula III.

Experimental

Lupulone was prepared by extraction of hop cones with petroleum ether, 30–60°, crystallization from the concentrated extract followed by recrystallization from petroleam ether and finally several times from 90% methanol. It had the m.p. 92–93°.

Humulone was isolated from the mother liquors of the petroleum ether extract of hop cones, after crystallization of lupulone, as the *o*-phenylenediamine complex. This was recrystallized three times from benzene–petroleum ether (1:2) to the constant rotation, $[\alpha]^{26}\text{D}$ −162° in dioxane and −124° in absolute ethanol. Humulone was then isolated from the *o*-phenylenediamine complex in the usual manner.[9]

Ozonization of Humulone.—Ozonized oxygen was bubbled through a solution of 1.15 g. (0.00317 equiv.) of humulone in

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[6] Wieland's failure to isolate acetone as the dinitrophenylhydrazone was probably due to the fact that from mixtures in ethanol of dinitrophenylhydrazones of acetone and isobutyraldehyde, the latter tends to crystallize first and accordingly the acetone derivative will accumulate in the mother liquor. Its presence is difficult to show except by chromatography.

[7] All yields are based on the pure crystalline dinitrophenylhydrazones.

[8] An outstanding example of this is to be found in the report of Hey, Honeyman and Peal, *J. Chem. Soc.*, 2881 (1950). These investigators observed that when fucosterol was ozonized for ten minutes, a 76% yield of acetaldehyde was obtained while ozonization for three hours yielded no acetaldehyde.

[9] W. Wöllmer, *Ber.*, **49**, 780 (1916).

30 ml. of glacial acetic acid for one hour at 20–22°. Absorption of ozone was quantitative during the first 50 minutes and the rate of absorption rapidly decreased during the next ten minutes. The ozonide was decomposed by the addition of 5 g. of zinc dust and 10 ml. of water. After standing 20 minutes, the solution was separated from the zinc by filtration into a 250-ml. flask. The ozone reaction flask was rinsed with 100 ml. of water, which was added to the filtrate. The solution was distilled to approximately one-third of its original volume and the distillate was collected in a suspension of 1.4 g. of 2,4-dinitrophenylhydrazine hydrochloride in 50 ml. of 2 *N* hydrochloric acid. The suspension of dinitrophenylhydrazones was extracted with 500 ml. of benzene, the benzene solution dried over sodium sulfate and concentrated to 150 ml. when it was ready for chromatography.

Chromatography of the Dinitrophenylhydrazones from Ozonized Humulone.—Silicic acid, Mallinckrodt 100 mesh Analytical Reagent[10] was washed on a Buchner funnel successively with 3 vols. of absolute ethanol, 3 vols. of acetone and 3 vols. of petroleum ether 30–60° and dried 18 hours at 110–115° and 25 mm. A column of the silicic acid, 52 mm. × 220 mm., was prepared and prewashed with one bed volume of anhydrous ethyl ether followed by three volumes of hexane.

One-half of the benzene solution (75 ml.) of dinitrophenylhydrazones from ozonized humulone was diluted with two volumes of hexane and adsorbed on the column. Development with hexane–ether (6:1) yielded an intense slow-moving band and a second extremely faint fast-moving zone. The fast zone, on elution, yielded approximately 5 mg. of orange material. This was not isobutyraldehyde dinitrophenylhydrazone as shown by a mixed chromatogram with authentic isobutyraldehyde dinitrophenylhydrazone. From the principal band there was obtained a crystalline product which on recrystallization from 35 ml. of absolute ethanol yielded 638 mg. (126 mole %). The identity of this compound as acetone 2,4-dinitrophenylhydrazone was demonstrated by mixed capillary melting points with the authentic compound, m.p. 124–125°, by microscopic observation of fusion behavior, and comparison of optical and crystallographic properties with the authentic compound. The derivative had the analyses: Found: C, 45.6; H, 4.24; N, 23.3. Calculated for $C_9H_{10}N_4O_4$; C, 45.38; H, 4.23; N, 23.53.

Ozonization of Lupulone.—Lupulone, 1.159 g. (0.0028 equiv.) was ozonized under the same conditions as humulone for one hour. One-half of the dinitrophenylhydrazone solution when chromatographed as above yielded a slow, sharp intense band and a very faint fast-moving zone. The fast band yielded only a trace of yellow resinous material and the main band yielded 885 mg. (198 mole %) of acetone dinitrophenylhydrazone. The absorption of ozone was practically quantitative during the one-hour period. Ozonization of 1.18 g. (0.00285 equiv.) of lupulone for two hours yielded 1.418 g. of acetone dinitrophenylhydrazone (209 mole % yield).

Isolation of Methyl *n*-Propyl Ketone Dinitrophenylhydrazone.—Lupulone, 923 mg. (0.00223 equiv.) in 30 ml. of acetic acid, was ozonized at 20–22° for six hours. The ozonide was decomposed and the dinitrophenylhydrazone of the distillate prepared as previously described. An aliquot of the benzene solution of dinitrophenylhydrazones amounting to 64% of the total was adsorbed on a silicic acid column 52 mm. × 210 mm. Development with hexane–ether (6:1) yielded two slow-moving closely spaced bands in approximately the expected position for acetone dinitrophenylhydrazone and a fast-moving band in the expected position for the isobutyraldehyde derivative. Each of the slow-moving bands yielded traces of unidentifiable crystalline material. The fast band yielded 46 mg. (12 mole %) of the 2,4-dinitrophenylhydrazone of methyl *n*-propyl ketone crystallized from methanol. No other material could be obtained from the mother liquor. The identity of this material was established by mixed melting point with authentic methyl *n*-propyl ketone 2,4-dinitrophenylhydrazone, m.p. 145–147°, microscopic observation of fusion behavior, and by comparison of optical and crystallographic properties.

Acknowledgment.—The author expresses his appreciation to Mr. L. M. White for analysis, to Mr. Gordon Alderton for a supply of lupulone, to Dr. F. T. Jones for microscopic identifications, and to Dr. T. W. Campbell for the use of an ozone apparatus.

ALBANY 6, CALIF. RECEIVED FEBRUARY 22, 1951

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[10] Mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroidal Sapogenins. X.[1a] Unsaturated 22-Isospirosten-3-ones[1b,2]

BY R. YASHIN, G. ROSENKRANZ AND CARL DJERASSI

$\Delta^{5,7}$-22-Isospirostadien-3β-ol (I) on Oppenauer oxidation leads to $\Delta^{4,7}$-22-isospirostadien-3-one (II), only one double bond migrating into conjugation with the carbonyl group. Similar treatment of $\Delta^{5,7,9(11)}$-22-isospirostatrien-3β-ol (IV) affords $\Delta^{4,7,9(11)}$-22-isospirostatrien-3-one (V), which upon catalytic reduction in an alkaline medium yields $\Delta^{7,9(11)}$-22-isospirostadien-3-one (VIII) and thence, by lithium aluminum hydride reduction, $\Delta^{7,9(11)}$-22-isospirostadien-3α-ol (IX). These two dienes (VIII and IX) of the 5-*normal* configuration represent important intermediates for the chemical introduction of an oxygen atom into position C-11. Conversion of $\Delta^{4,7,9(11)}$-22-isospirostrien-3-one (V) to the enol acetate VI followed by acid hydrolysis gives $\Delta^{4,6,8}$-22-isospirostatrien-3-one (VII). Dibromination of Δ^{4}-22-isospirosten-3-one (XI) and dehydrobromination of the resulting 2,6-dibromo-Δ^{4}-3-ketone XII with collidine affords $\Delta^{1,4,6}$-22-isospirostatrien-3-one (XIII). Ultraviolet and infrared spectroscopic data are reported for all products and the infrared band at 1656 cm.$^{-1}$ is shown to be associated with a conjugated trienone system (VII, X, XIII).

As pointed out in earlier communications,[3,4] 7,8;9,11-dienes of the steroid series represent useful intermediates for the chemical introduction of an oxygen atom into position C-11 of ring C unsubstituted starting materials. The preparation of certain 7,8;9,11-dienes of the sapogenin series[3] and thence, by side chain degradation, of the pregnane series[4] was accomplished in a facile manner, since they possessed the *allo* configuration (rings A/B *trans*) which is produced almost exclusively on hydrogenation of 5,6;7,8-dienes (I), the starting materials employed. The synthesis of 7,8;9,11-dienes with the normal configuration (rings A/B *cis*) (*e.g.*, VIII) appeared particularly attractive, since such dienes are closely related to the 9,11-

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[1] (a) IX, A. Sandoval, J. Romo, St. Kaufmann, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3820 (1951); (b) taken in part from a thesis presented by Srta. Rosa Yashin to the Facultad de Química, Universidad Motolinia.

[2] For nomenclature of steroidal sapogenins, see G. Rosenkranz and C. Djerassi, *Nature*, **166**, 104 (1950).

[3] G. Rosenkranz, J. Romo, E. Batres and C. Djerassi, *J. Org. Chem.*, **16**, 298 (1951).

[4] C. Djerassi, J. Romo and G. Rosenkranz, *ibid.*, **16**, 754 (1951).