[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Terpenoids. XIII.[1] The Structures of the Cactus Triterpenes Machaeric Acid and Machaerinic Acid[2]

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Machaeric acid, a new triterpene acid recently isolated from the cactus *Machaerocereus gummosus*, has been shown to have the structure 21-keto-oleanolic acid by appropriate correlation with a 21-dehydro-oleanolic acid derivative obtainable from the triterpene rehmannic acid. Machaerinic acid, a triterpene acid present in trace amounts in the same cactus is one of the two possible isomeric 21-hydroxy-oleanolic acids as demonstrated by sodium borohydride reduction of machaeric acid. These two acids are the first examples of naturally occurring triterpenes with oxygen functions at position 21.

The genus *Machaerocereus* of the *Cactaceae* family is limited to only two species, *M. gummosus* and *M. eruca*. Both of them have been examined in this Laboratory[4,5] and the structure of the principal neutral triterpene, gummosogenin (V), from *M. gummosus* has been elucidated.[4] The present paper is concerned with the structure proof of the acidic triterpenes of this cactus.[6]

Chromatography of the methyl esters of the acidic triterpene fraction of *M. gummosus* furnished[5] a pure methyl ester, $C_{31}H_{48}O_4$, which was characterized further as the monoacetate and the free acid ($C_{30}H_{46}O_4$). We have named the latter machaeric acid. The infrared spectrum of methyl machaerate (Ib) indicated that the fourth oxygen atom was present as a carbonyl function ($\lambda_{\max}^{CHCl_3}$ 5.87 μ) and this could be confirmed by the ready formation of a semicarbazone. The Wolff–Kishner reduction (Huang-Minlon modification[7]) furnished oleanolic acid (IIa) while lithium aluminum hydride reduction led to a triol (IVa) which formed a triacetate (IVb). Machaeric acid is thus *x*-oxo-oleanolic acid with the type and location of the carbonyl group to be determined.

Methyl machaerate (Ib) does not reduce Tollens reagent and the corresponding acetate (Ic) is stable to chromium trioxide. These observations and the dehydration experiment described below eliminate an aldehyde formulation. Oxidation of methyl machaerate (Ib) with the chromium trioxide–pyridine reagent[8] yielded a diketone III which was stable to base, did not show any coloration with ferric chloride and exhibited no high selective ultraviolet absorption, thus eliminating any position in ring A as the location for the unknown carbonyl group. Positions 16 and 22 are excluded because the isolation[5] of machaeric acid involved the acid hydrolysis of the glycoside mixture and such treatment would have caused decarboxylation of any β-keto esters present. The reactivity of the carbonyl group (ready semicarbazone formation) precludes positions 6, 11[9] and 19, thus leaving only carbon atoms 7, 15 and 21 for consideration. Methyl machaerate does not condense with ethyl formate under standard conditions, indicating the fairly hindered nature of the adjacent methylene group (C-6, 16 or 21). An unambiguous solution of this structural problem was provided as follows.

Sodium borohydride reduction of methyl acetylmachaerate (Ic) gave the corresponding methyl *x*-hydroxyacetyloleanolate (VIb), which could be dehydrated with phosphorus oxychloride in pyridine or phosphorus pentachloride in hexane solution to yield methyl anhydroacetyloleanolate. That no skeletal rearrangement had occurred during the dehydration was demonstrated by the catalytic hydrogenation of the anhydro derivative to methyl acetyloleanolate (IIc). The anhydro derivative was not identical with methyl acetylanhydrosumaresinolate[10] or methyl acetyl-15-dehydro-oleanolate[11] thus definitely excluding position 15 and conceivably also position 7[12] from consideration as points of attachment for the carbonyl group of machaeric acid.

Saponification of the dehydration product VIIb followed by chromium trioxide oxidation of the resulting methyl anhydromachaerate (VIIa) led to $\Delta^{12,21}$-18β-oleadien-3-one-28-oic acid methyl ester (VIII), which was shown to be identical with an authentic sample prepared by thermal treatment of methyl rehmannate (IX).[13] Machaeric acid is, therefore, 21-keto-oleanolic acid (Ia) and represents the first naturally occurring triterpene for which the presence of an oxygen function at C-21 has been demonstrated.

A point worth noting is the unusual behavor of methyl machaerate during saponification.[5] In contrast to methyl oleanolate (IIb) and other related triterpenes in which the esters are very difficult to saponify (diethylene glycol or sealed tube), methyl machaerate is saponified completely by refluxing with 5% methanolic potassium hydroxide. While the activating influence of a keto group in γ-

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[1] Paper XII, C. Djerassi and G. H. Thomas, *Chem. & Ind.*, 1354 (1954).

[2] We are greatly indebted to the Rockefeller Foundation for financial support of this investigation.

[3] Postdoctorate Research Fellow, 1953–1954.

[4] C. Djerassi, L. E. Geller and A. J. Lemin, *Chem. & Ind.*, 161 (1954); THIS JOURNAL, **76**, 4089 (1954).

[5] C. Djerassi, L. H. Liu, E. Farkas, A. E. Lippman, A. J. Lemin, L. E. Geller, R. N. McDonald and B. J. Taylor, *ibid.*, **77**, 1200 (1955).

[6] A part of this work has been announced in a preliminary communication (C. Djerassi and A. E. Lippman, *Chem. & Ind.*, 960 (1954)).

[7] Huang-Minlon, THIS JOURNAL, **71**, 3301 (1949).

[8] G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

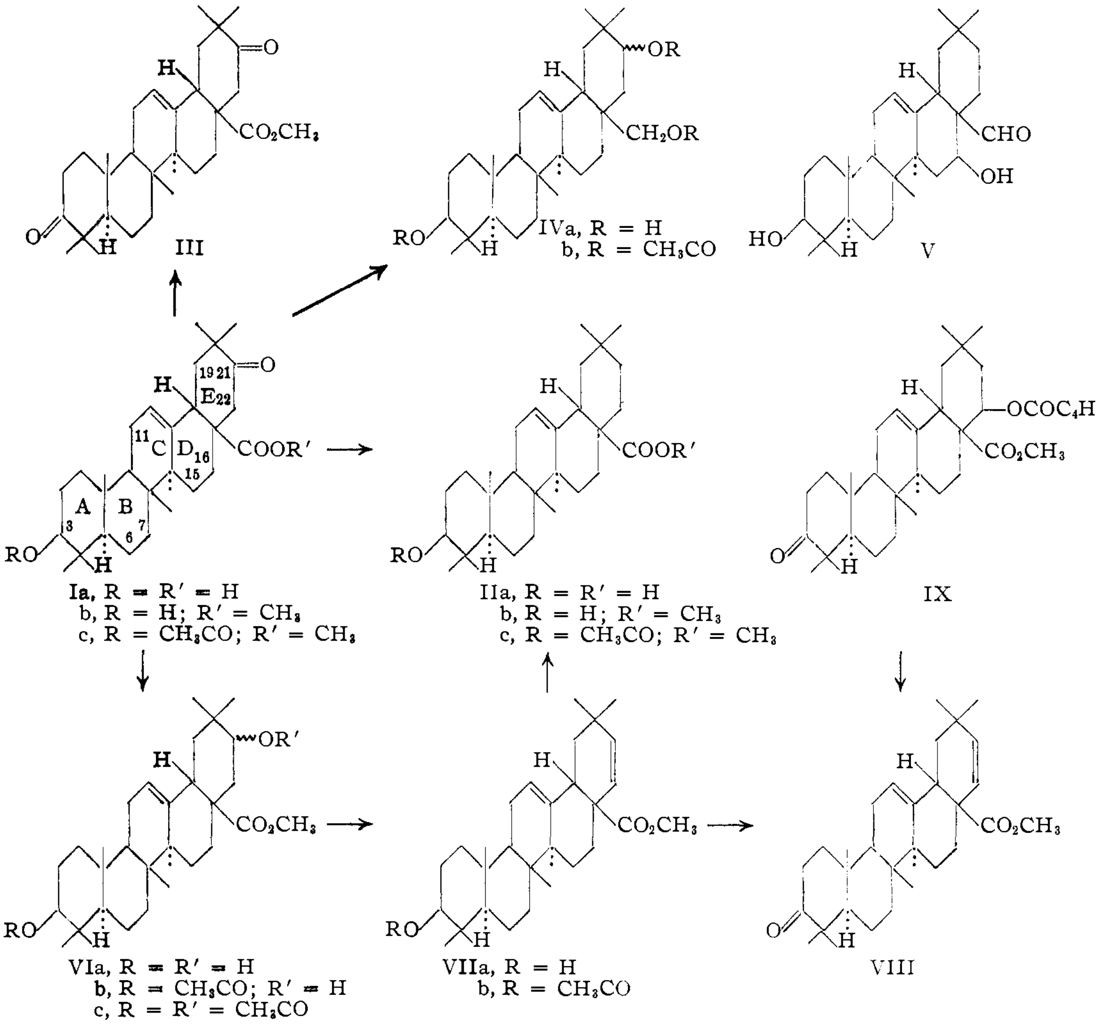
[9] This position also is excluded on the basis of the spectroscopic data which do not show any α,β-unsaturated keto moiety.

[10] We are grateful to Dr. O. Jeger (E. T. H., Zürich) for a sample of this substance prepared from sumaresinolic acid (L. Ruzicka, O. Jeger, A. Grob and H. Hösli, *Helv. Chim. Acta*, **26**, 2283 (1943)).

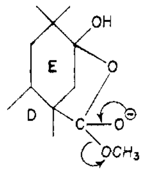
[11] This sample, prepared from echinocystic acid, was kindly provided by Prof. C. R. Noller (Stanford University) (*cf.* D. Frazier and C. R. Noller, THIS JOURNAL, **66**, 1267 (1944)).

[12] The element of doubt in this instance arises from the fact that the double bond in anhydrosumaresinolic acid could be at 5–6 or 6–7.

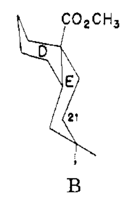
[13] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 900 (1954). We are indebted to Prof. D. H. R. Barton (Birkbeck College) for the comparison sample.



keto-esters can be explained readily as being due to an intermediate hemiacetal such as A, this would appear to be impossible on steric grounds in the usual all-chair conformation attributed to triterpenes (B) of the 18β-series (rings D/E *cis*) and hence if an intermediate such as A is operative, it would require a change in the conformation of ring D.



A



B

From the accumulated methyl ester mother liquors originating from over 80 kg. of cactus, there was isolated approximately 100 mg. of a new methyl ester ($C_{31}H_{50}O_4$), containing two more hydrogen atoms than methyl machaerate, and which was named methyl machaerinate. The substance readily formed a diacetate and upon chromium trioxide oxidation was transformed into methyl 3-ketomachaerate (III). It is clear, therefore, that machaerinic acid must be a Δ^{12}-3,21-dihydroxy-18β-oleanen-28-oic acid with unknown configuration at C-3 and C-21. That the configuration at C-3 is identical with that of oleanolic and machaeric acids (3β) was demonstrated by partial synthesis from the latter triterpene. Saponification of the sodium borohydride reduction product VIb of methyl acetylmachaerate and careful chromatography of the diol mixture resulted in the separation of two epimeric diols VIa, each of which gave a diacetate VIc after acetylation at room temperature. One of the diols and its derived diacetate were shown to be identical with methyl machaerinate and its diacetate, thus defining methyl machaerinate as methyl 21ξ-hydroxyoleanolate (VIa). Insufficient evidence is available to assign a definite configuration to the 21-hydroxyl function.[14]

Experimental[15]

Methyl Machaerate Semicarbazone.—Semicarbazide hydrochloride (250 mg.) and sodium acetate (350 mg.) were ground in 2.5 cc. of ethanol, filtered and the filtrate was refluxed with 100 mg. of methyl machaerate (Ib)[5] for 2 hours. The mixture was poured into water, the precipitate was collected and triturated successively with methanol, acetone, ethyl acetate and chloroform, whereupon it decomposed at 290°.

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[14] Calculation of molecular rotation differences (cf. W. Klyne and W. M. Stokes, *J. Chem. Soc.*, 1979 (1954)) would suggest that the configuration is β.

[15] All melting points were determined on the Kofler block. Rotations were measured in chloroform solution. We are indebted to Geller Laboratories, Hackensack, N. J., for the microanalyses.

Anal. Calcd. for $C_{32}H_{51}N_3O_4$: C, 70.94; H, 9.49; N, 7.76. Found: C, 70.73; H, 9.24; N, 7.74.

Wolff–Kishner Reduction[7] **of Methyl Machaerate (Ib).**—The ester (100 mg.) was refluxed for one hour with 3 cc. of absolute ethanol, 8 cc. of diethylene glycol and 0.34 cc. of 85% hydrazine hydrate. Solid potassium hydroxide (40 mg.) was then added, refluxing was continued for an additional hour after which the condenser was removed and the temperature of the boiling reaction mixture was allowed to rise to 190°. The condenser was replaced and the mixture was refluxed for 2.5 hours. After cooling, it was poured into dilute hydrochloric acid, extracted with ether, washed, dried and concentrated until crystals appeared. Filtration afforded 41 mg. of oleanolic acid (IIa), which did not depress the m.p. of an authentic sample but, for adequate characterization, the acid was esterified with diazomethane and the methyl ester purified by chromatography and recrystallization from methanol. Methyl oleanolate (IIb), m.p. 198–200°, $[\alpha]^{20}\mathrm{D}$ +76° was identified by mixture melting point and infrared comparison.

Anal. Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 78.86; H, 10.69.

Lithium Aluminum Hydride Reduction of Methyl Machaerate (Ib).—A solution of 400 mg. of methyl machaerate in 20 cc. of tetrahydrofuran was stirred at room temperature for 4.5 hours with 1 g. of lithium aluminum hydride in 100 cc. of anhydrous ether. After addition of a saturated sodium sulfate solution, the mixture was dried with anhydrous sodium sulfate and the filtered organic layer was evaporated to dryness to yield 250 mg. of crystals, m.p. 273–276°. Successive recrystallizations from glacial acetic acid furnished the analytical sample of the triol IVa, m.p. 293.5–295°.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.24; H, 10.87.

The triacetate (IVb), prepared by the acetic anhydride–perchloric acid procedure, was purified by chromatography and recrystallization from methanol; m.p. 214–215°, $[\alpha]^{20}\mathrm{D}$ +2°.

Anal. Calcd. for $C_{36}H_{56}O_6$: C, 73.93; H, 9.65. Found: C, 73.73; H, 9.91.

Δ^{12}-18β-Oleanen-3,21-dione-28-oic Acid Methyl Ester (III).—Methyl machaerate (60 mg.) was oxidized with 61 mg. of chromium trioxide in 1 cc. of pyridine for 9 hours at room temperature. Two crystallizations from methanol yielded 34 mg. of diketone, m.p. 181–189°. The analytical sample was obtained from acetone–hexane, m.p. 188–191°, $[\alpha]^{24}\mathrm{D}$ +43°, $\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.80 and 5.90 μ, no high selective ultraviolet absorption, no color with alcoholic ferric chloride solution. The diketone was recovered unchanged after 17 hours at room temperature in 5% methanolic sodium hydroxide solution.

Anal. Calcd. for $C_{31}H_{46}O_4$: C, 77.13; H, 9.61. Found: C, 76.97; H, 9.82.

Oxidation of methyl machaerinate (VIa) to the same diketone III was carried out as above except that the amount of chromium trioxide and the reaction time was doubled.

Δ^{12}-18β-Oleanene-3β,21ξ-diol-28-oic Acid Methyl Ester 3-Monoacetate (VIb).—To 920 mg. of methyl acetylmachaerate (Ic)[5] in 10 cc. of dioxane was added 2.45 g. of sodium borohydride in 10 cc. of the same solvent. After one hour at room temperature, excess 10% hydrochloric acid was added and the mixture was extracted with ether. Crystallization of the residue from acetone–hexane yielded 655 mg. of the monoacetate VIb, m.p. 201–209°, which was used for the dehydration experiments. Further recrystallization from methanol and acetone–hexane gave material with m.p. 214–216°, $[\alpha]^{24}\mathrm{D}$ +69°.

Anal. Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91; CH_3CO, 8.14. Found: C, 74.72; H, 9.89; CH_3CO, 7.78.

The above material is probably fairly homogeneous (see separation of diol isomer below) and belongs to the same diastereoisomeric series as machaerinic acid since acetylation of 62 mg. of the above reduction product (acetic anhydride–pyridine, room temperature, 25 hours), yielded 39 mg. of analytically pure **diacetate (VIc)**, which proved to be identical (infrared comparison) with methyl diacetylmachaerinate, m.p. 278–280°, $[\alpha]^{23}\mathrm{D}$ +85°.

Anal. Calcd. for $C_{35}H_{54}O_6$: C, 73.64; H, 9.54. Found: C, 73.69; H, 9.45.

$\Delta^{12,21}$-18β-Oleadien-3β-ol-28-oic Acid Methyl Ester Acetate (VIIb).—The above sodium borohydride reduction product VIb (310 mg.) was left at room temperature for 11 hours with 2 cc. of phosphorus oxychloride and 10 cc. of pyridine and then heated (steam-bath) for one hour. Dilution with water, ether extraction and chromatography on 10 g. of alumina furnished 220 mg. of the dehydration product which after several recrystallizations from hexane and from methanol exhibited m.p. 170–174°, $[\alpha]^{22}\mathrm{D}$ +17°, strong yellow color with tetranitromethane. Essentially the same yield of the dehydration product was obtained when 270 mg. of VIb in 120 cc. of purified hexane was stirred at room temperature for 2 hours with 300 mg. of phosphorus pentachloride, a stream of dry nitrogen being bubbled continuously through the solution.

Anal. Calcd. for $C_{33}H_{50}O_4$: C, 77.60; H, 9.87. Found: C, 77.46; H, 9.89.

Saponification with 5% methanolic potassium hydroxide (1 hour, steam-bath) and recrystallization from methanol gave long, colorless needles of **$\Delta^{12,21}$-18β-oleadien-3β-ol-28-oic acid methyl ester (VIIa)**, m.p. 184–185°, $[\alpha]^{24}\mathrm{D}$ −2°.

Anal. Calcd. for $C_{31}H_{48}O_3$: C, 79.43; H, 10.32. Found: C, 79.23; H, 10.24.

Hydrogenation of $\Delta^{12,21}$-18β-Oleadien-3β-ol-28-oic Acid Methyl Ester Acetate (VIIb) to Methyl Acetyloleanolate (IIc).—A solution of 35 mg. of the dehydration product VIIIb in 15 cc. of glacial acetic acid was shaken in an atmosphere of hydrogen with 150 mg. of platinum oxide for 16 hours at room temperature and atmospheric pressure. Filtration of the catalyst, evaporation of the filtrate to dryness *in vacuo* and crystallization of the residue from acetone yielded 27 mg. of methyl acetyloleanolate (IIc), m.p. 217–218°, $[\alpha]^{23}\mathrm{D}$ +68°. Identity was established by mixture melting point determination and infrared comparison.

Anal. Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.07; H, 10.09.

$\Delta^{12,21}$-18β-Oleadien-3-one-28-oic Acid Methyl Ester (VIII).—The saponified dehydration product VIIa (54 mg.) was oxidized in the usual manner with 51 mg. of chromium trioxide in 2 cc. of pyridine for 10.5 hours at room temperature. Two recrystallizations of the oxidation product from methanol led to 21 mg. of the desired 3-keto methyl ester VIII, m.p. 120–124°, undepressed on admixture with an authentic sample[13] (same melting point on our apparatus), $[\alpha]^{24}\mathrm{D}$ +17°.

Anal. Calcd. for $C_{31}H_{46}O_3$: C, 79.78; H, 9.94. Found: C, 80.36; H, 9.95.

Isolation of Methyl Machaerinate (VIa).—The total ether-eluted methyl ester fraction (2.0 g.)[6] originating from the extraction of *ca.* 80 kg. of the cactus *Machaerocereus gummosus* was rechromatographed on 300 g. of alumina deactivated with 12 cc. of 10% acetic acid. Elution with benzene–ether (9:1) gave 660 mg. of crude methyl machaerate (Ib) while from the later eluates (same solvents) there was obtained 800 mg. of material which after eleven recrystallizations from methanol, acetone–hexane and ether melted constantly at 232–234°, $[\alpha]^{31}\mathrm{D}$ +76°; the analytical sample was sublimed at 210° and 0.08 mm.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36; CH_3O, 6.36. Found: C, 76.17; H, 10.07; CH_3O, 6.26.

Acetylation of 50 mg. of methyl machaerinate at room temperature with acetic anhydride–pyridine yielded 45 mg. of methyl **diacetylmachaerinate (VIc)**, m.p. 278–280°, $[\alpha]^{27}\mathrm{D}$ +86°, which was shown to be identical with the acetylated sodium borohydride reduction product of methyl acetylmachaerate by mixture melting point and infrared comparison.

Sodium Borohydride Reduction of Methyl Acetylmachaerate (Ic). Separation of Isomers.—The crude reaction product from the reduction of 610 mg. of methyl acetylmachaerate (Ic) with 530 mg. of sodium borohydride in 25 cc. of dioxane (for details see above) was saponified by refluxing for one hour with 1% methanolic potassium hydroxide. The neutral reaction product, dissolved in benzene, was chromatographed on 60 g. of neutral alumina (activity III). Seventeen fractions eluted with benzene–ether (9:1) weighed 240 mg. and after recrystallization from methanol gave 170 mg. of one of the isomers of the diol VIa, m.p. 274–275°, $[\alpha]^{27}\mathrm{D}$ +64°.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.21; H, 10.10.

Acetylation of 88 mg. of the diol with acetic anhydride–pyridine at room temperature furnished 79 mg. of the corresponding **diacetate**, m.p. 252–259°, $[\alpha]^{26}D$ +31°.

Anal. Calcd. for $C_{35}H_{54}O_6$: C, 73.64; H, 9.54. Found: C, 73.32; H, 9.51.

The combined ether eluates weighed 256 mg. and after recrystallization from methanol gave 175 mg. of methyl machaerinate (VIa) (infrared comparison and undepressed mixture melting point) m.p. 231–234°, $[\alpha]^{27}D$ +78°. Acetylation (room temperature) of 70 mg. of this material led, after one recrystallization from ether, to 66 mg. of methyl diacetylmachaerinate (VIc), m.p. 278–280°; identity was established in the usual manner by mixture melting point and infrared comparison.

Attempts to establish a difference in the reactivity of the isomeric diols through the corresponding cathylates[16] were unsuccessful.

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[16] L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero and T. Utne, THIS JOURNAL, **74**, 3309 (1952).

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, FACULTY OF SCIENCE, CAIRO UNIVERSITY]

4-Styrylcoumarins and 2,3-Dimethylquinoxaline in Diene Syntheses

BY AHMED MUSTAFA AND MOHAMED KAMEL

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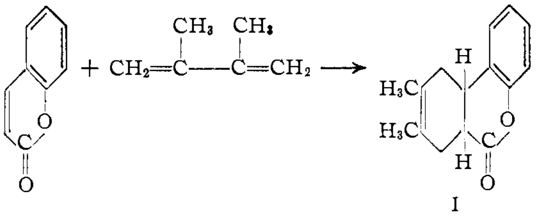
Derivatives of 6H-dibenzo(b,d)pyran (III), 5(6H)-oxachrysene (V) and hexahydrophenazine (XIa or XIb) may be obtained from 4-styrylcoumarins (II and IV) and 2,3-dimethylquinoxaline (IX) in the Diels–Alder reactions. An improved method for the preparation of 4-styrylcoumarins, in satisfactory yields, is described.

Coumarin functions as a dienophile in the Diels–Alder synthesis.[1] It reacts with 2,3-dimethylbutadiene only under forcing conditions (260°) and in the presence of a large excess of this reagent to give poor yields of 8,9-dimethyl-6a,7,10,10a-tetrahydrodibenzopyrone (I).

The use of 4-styrylcoumarins as diene components in the Diels–Alder reaction seems not to have been investigated.[2] We have now found that, in the heterocyclic series, the oxygen-containing 4-styryl-7-methylcoumarins (II) and 4-styryl-7,8-benzocoumarins (IV), having conjugated double bonds, one of which is part of the heterocyclic ring, show the diene behavior. They add dienophile components such as maleic anhydride. Thus, an easy formation of the 6H-dibenzo(b,d)pyran derivatives (III) is available, *e.g.*, IIIa is obtained when 4′-methoxy-4-styryl-7-methylcoumarin (IId) is allowed to react with maleic anhydride in boiling xylene. Similar results, leading to IIIb, were observed in the case of 4′-hydroxy-4-styryl-7-methylcoumarin (IIc).



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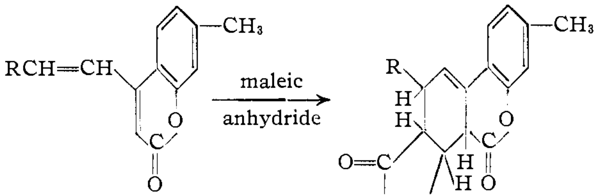
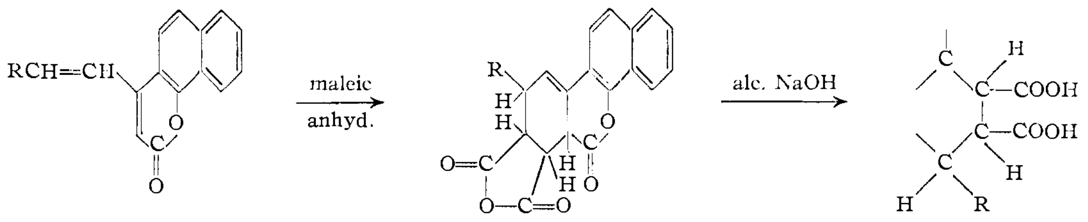


IIa, R = C_6H_5
b, R = p-$C_6H_4CH_3$
c, R = p-C_6H_4OH
d, R = p-$C_6H_4OCH_3$
e, R = 4′,3′-$C_6H_3(OH)OCH_3$
f, R = 3′,4′-$C_6H_3{:}O_2CH_2$

IIIa, R = p-$C_6H_4OCH_3$
b, R = p-C_6H_4OH

Similar reactions were carried out with 4-styryl-7,8-benzocoumarins (IVa, IVc and IVe), leading to derivatives of 5(6H)-oxachrysene (V), namely, Va–Vc, respectively.

The adducts obtained by these Diels–Alder syntheses are practically colorless and are insoluble in alkali. Treatment of adduct Vb with alcoholic sodium hydroxide and subsequent acidification yielded the corresponding alkali-soluble acid which was proved to be identical with the product obtained by the action of maleic acid on IVc. Treatment of the adduct acid with acetic anhydride regenerated Vb.



IVa, R = C_6H_5
b, R = p-$C_6H_4CH_3$
c, R = p-$C_6H_4OCH_3$
d, R = 4′,3′-$C_6H_3(OH)OCH_3$
e, R = 3′,4′-$C_6H_3{:}O_2CH_2$

Va, R = C_6H_5
b, R = p-$C_6H_4OCH_3$
c, R = 3′,4′-$C_6H_3{:}O_2CH_2$

VI
(abbreviated formula)

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[1] R. Adams, W. D. McPhee, R. B. Carlin and Z. W. Wicks, THIS JOURNAL, **65**, 356 (1943); R. Adams and R. B. Carlin, *ibid.*, **65**, 360 (1943).

[2] *Cf.* The diene behavior of 2-styrylchromones toward dienophiles, *e.g.* maleic anhydride (A. Schönberg, A. Mustafa and G. Aziz, *ibid.*, **76**, 4576 (1954).