Structure of Pristimerin and Celastrol[1]

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Pristimerin is an antibiotic isolated by Bhatnagar and Divekar[2] from the plant *Pristimera indica* (Wild) A. C. Smith, syn. *Hippocratea indica* Wild (*Celastraceae*), which besides being active against the common gram-positive organisms, is characterized by its activity against the *Viridans* group of streptococci. It has been found to be an effective chemotherapeutic agent in the treatment of tonsilitis, inflammation of the naso-pharyngeal mucosa, etc. Celastrol is the red pigment obtained from the root bark of *Celastrus scandens*[3,4]; tripterine from *Tripterygium wilfordii* Hook f. is also reported to be identical with celastrol[5]. In the present paper we wish to present structure (I) for pristimerin and (I′) for celastrol, and also to make comments on structural studies so for undertaken[3,6,7,8] on these substances.

1) The present studies were commenced in the laboratory of Professor L.F. Fieser, Harvard University, U.S.A. A communication has appeared in *J. Am. Chem. Soc.*, **77**, 3915 (1955).

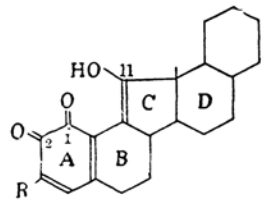
2) S.S. Bhatnagar and P.V. Divekar, *J. Sci. Industr. Res.*, **10B**, 56 (1951).

3) O. Gisvold, *J. Am. Chem. Soc.*, **28**, 449 (1939); **29**, 12 (1940); **31**, 529 (1942).

4) Chou and Mei, *Chinese J. Physiol.*, **10**, 529 (1936)

5) M.S. Schechter and H.L. Haller, *J. Am. Chem. Soc.*, **64**, 182 (1942).

6) L.F. Fieser and R.N. Jones, *J. Am. Pharm. Soc.*, **31**, 315 (1942).



(I) R=OCH_3
(I') R=OH

One isolated nuclear double bond, probably tri- or tetra-substituted.

$-C_7H_{15}$ (Containing two C-CH_3)

Pristimerin is obtained as orange needles and possesses one methoxyl and three C-methyl groups. A molecular formula of $C_{30}H_{40}O_4$ is in better accord with analysis than the previously assigned[7] formula of $C_{28}H_{36-8}O_4$. The fact that catalytic hydrogenation gave a colorless solution which gradually regained the original coloration when exposed to air, and that the *shape* of the ultraviolet curve (Fig. 1) was similar to *o*-quinones, e.g., 3-*n*-pentadecyl-1-,2-benzoquinone[9], 4-methyl-1,2-benzoquinone[10], 3-methoxy-1,2-benzoquinone[11] (Fig. 1), etc., suggested that two of the oxygens were contained in an *o*-benzoquinonoid arrangement. Though the infrared spectrum (Fig. 2a) was compared with those of several other *o*-quinones, the bands in the 6μ region are as yet not completely interpretable; it is now understood that the conjugated enol group is evidently involved. In spite of the 3μ hydroxyl band (*vide infra*) and a positive ferric chloride test, pristimerin was recovered unchanged from rather vigorous treatment with diazomethane. Acetylation under a variety of conditions did not afford a crystalline product, but the reductive acetylation proceeded smoothly to give a colorless optical-active[12] dihydro-diacetyl derivative. However, the ultraviolet spectrum of the reductive acetate (Fig. 1) showed no conspicuous maxima, excepting a shoulder-like peak at 278 mμ (log ε 2.90), which at most could only be attributed to a benzenoid nucleus. Support of the presence of an aromatic (i.e., benzenoid) ring was obtained from the infrared bands at 1775 and 1765 cm^{-1} (enol acetate), and at 1600 and 1515 cm^{-1} (aromatic) (Fig. 2b). The disappearance of the hydroxyl band, is also to be noted.

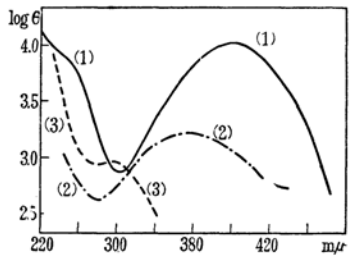


Fig. 1. ——— (1) Pristimerin
—·—· (2) 3-Methoxy-1,2-benzoquinone
---- (3) Reductive acetate

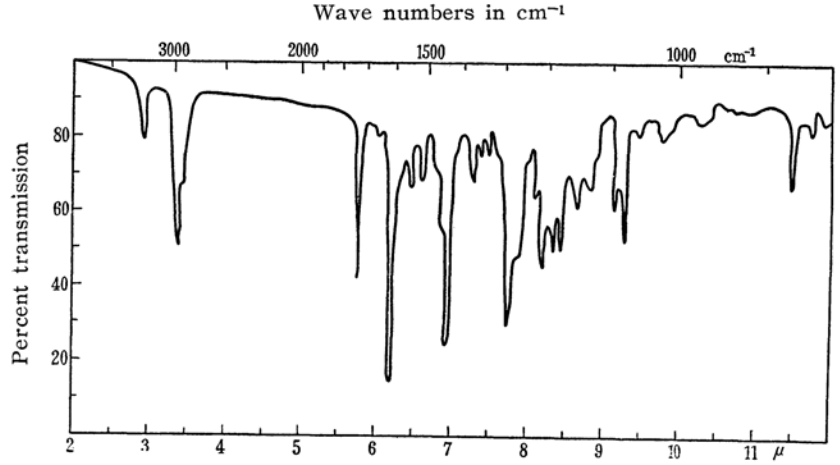


Fig. 2a. Pristimerin (CCl_4)

7) A.B.S. Kulkarni and R.C. Shah, *Nature*, **173**, 1237 (1954).
8) V.N. Kamat, F. Fernandes, and S.S. Bhatnagar, *J. Sci. Industr. Res.*, **14C**, 1 (1955).
9) H.S. Mason, *J. Am. Chem. Soc.*, **70**, 138 (1948).
10) S. Goldschmidt and F. Graef, *Ber.*, **61**, 1858 (1928).
11) Prepared according to the method of R. Willstätter and F. Müller, ibid., **44**, 2179 (1911).
12) The $[\alpha]_D$ of pristimerin could not be measured *owing to the coloration.*

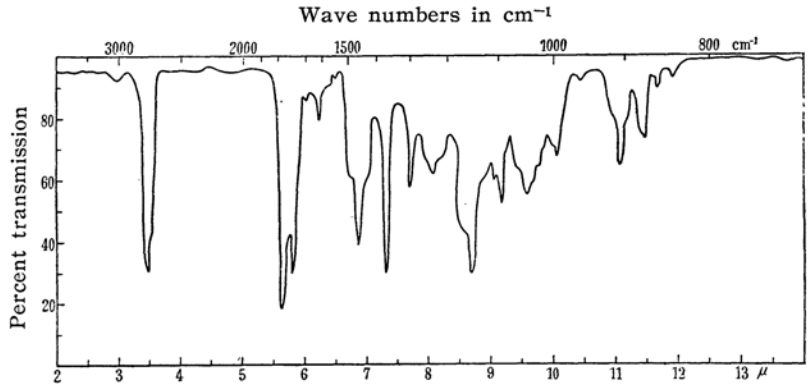


Fig. 2b. Reductive acetate (Chloroform)

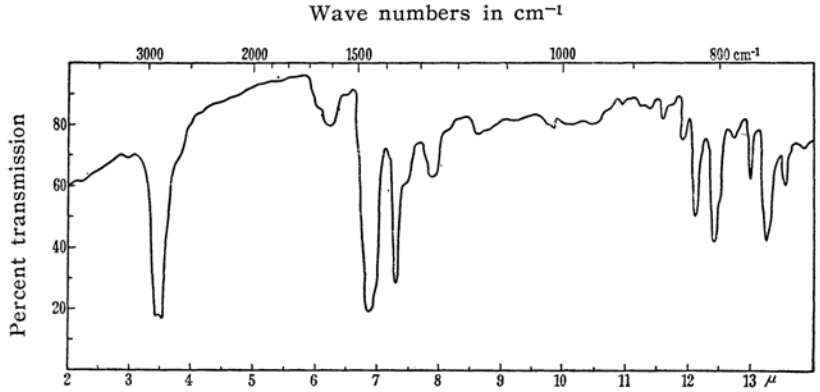


Fig. 2c. $C_{25}H_{22}$ (Nujol)

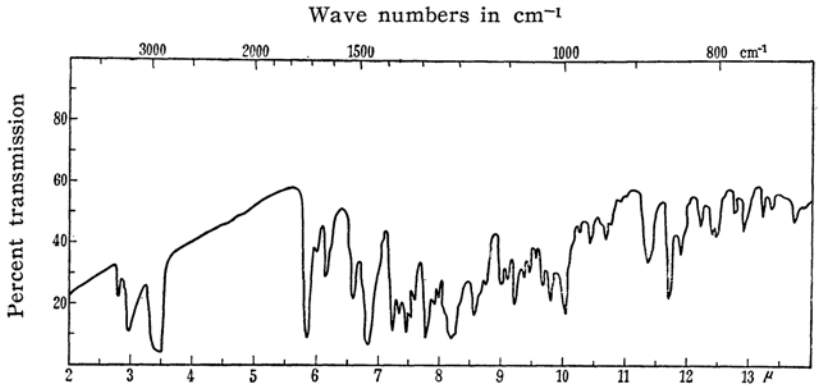


Fig. 2d. Pristimerol (Nujol)

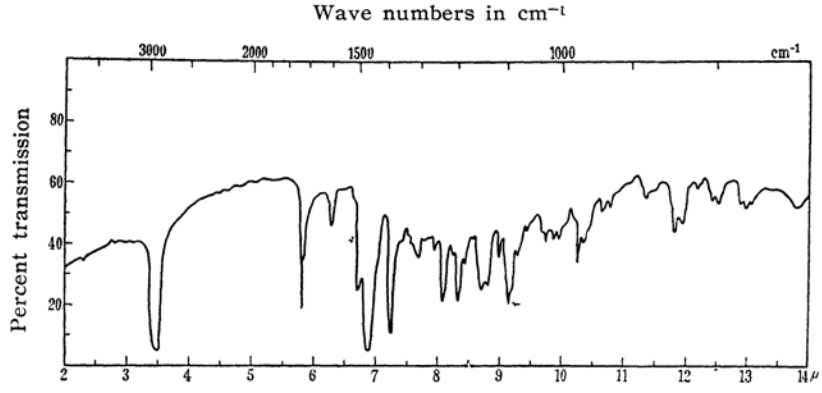


Fig. 2e. Pristimerol dimethyl ether (Nujol)

A micro zinc-dust distillation of pristimerin afforded, though in very poor yield, a strongly fluorescent crystalline product and a crude oil with an aromatic odor. The ultraviolet spectrum of the former (Fig. 3) was almost

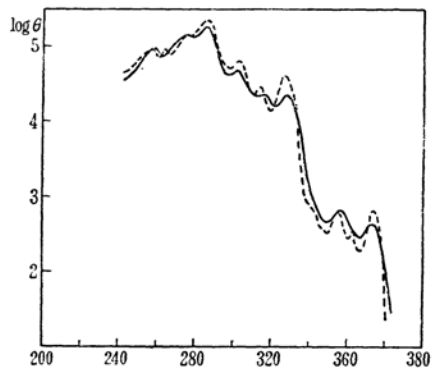


Fig. 3 ——— Zinc distliation product, $C_{23}H_{22}$
---- Picene

superimposable on that of picene except that the respective peaks were displaced slightly by 0–2 mμ towards longer wave-length; an infrared comparison clearly revealed the non-identity of the product with picene (infrared spectrum of product recorded in Fig. 2c[13]). The analysis checked with $C_{22}H_{23}$, i.e., a C_4H_9 substituted picene, and since bathochromic shifts of 5-7 mμ are observed in the ultraviolet spectra of dialkyl-substituted picenes as compared with the parent hydrocarbon[14], the product is suspected to be a monosubstituted picene with a C_4H_9-side chain. The spectrum was also comparable *in shape* with that of chrysene (and alkyl derivatives), and analysis equally agreed with a C_3H_7-substituted chrysene, $C_{21}H_{18}$, but in this case the bathochromic shifts of 6–17 mμ of the respective peaks are too high to be accounted for by three methyl groups. The crude brown oil possessed an ultraviolet spectrum somewhat similar to naphthalene derivatives. Although possibilities for ring formation during the zinc-dust distillation should not be overlooked, other evidences (*vide infra*) suggested the presence of rings A, B, and C, and also the five-membered nature of ring C; hence, a pentacyclic perhydro-1,2,7,8-dibenzofluorene skeleton with an angular methylgroup at the C/D ring junction has tentatively been assumed.

Treatment of pristimerin with sodium borohydride, or with boiling alcoholic hydrochloric acid yielded a colorless dihydro derivative, pristimerol, possessing a clear ultraviolet absorption (λ_{max}^{alc} 284 mμ, log ε 3.49, Fig. 4)

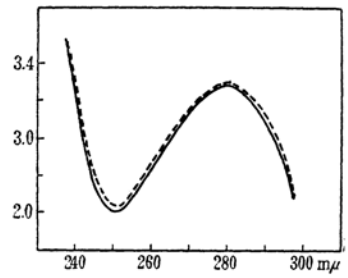


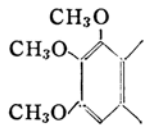
Fig. 4. ——— Pristimerol
---- 1,2,3,4-Tetrahydro-5,6,7-trimethoxy-naphthalene

which was of great value in subsequent studies. The original methoxyl group was retained, and two hydroxyl groups had been formed as evidenced by formation of a di-*p*-nitrobenzoate and a dimethyl ether (λ_{max}^{alc} 280 mμ, log ε 3.31); the infrared spectrum (Fig. 2d) showed that an hydroxylated ben-

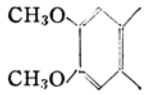
13) The authors are indebted to Research Laboratory, Baird Associates, Cambridge, U.S.A., for running micro-infrared spectra of the zinc distillation product and related compounds.

14) L. Ruzicka, et al., *Helv. Chim. Acta*, **19**, 377 (1936); **20**, 1155 (1937).

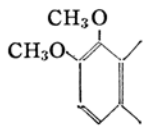
zenoid moiety was present in pristimerol. The disposition of the hydroxyl and methoxyl groups around the benzenoid ring was inferred in the following manner: At least two carbon side-chains should be attached to this ring to account for the condensed cyclic skeleton, and on the other hand, at least one position in the benzenoid ring is unsubstituted since pristimerol coupled with *p*-diazobenzenesulfonic acid. 1,4-Dihydroxybenzene and 1,2,4-trimethoxybenzene absorbed at 294 mμ (log ε 3.49) and 286 mμ (log ε 3.56), respectively, and attachment of two side-chains would shift the maxima to a wave-length longer than that of pristimerol or its dimethyl ether[15]; accordingly, absence of these groups is apparent. Two alkyl substituents attached to pyrogallol (λ_{max}^{alc} 267 mμ) or catechol (λ_{max}^{alc} 278 mμ)[16] may be expected to shift the absorption maximum to the region of 285 mμ: this would lead to the part structures (II), (III) and (IV) for pristimerol dimethyl ether.



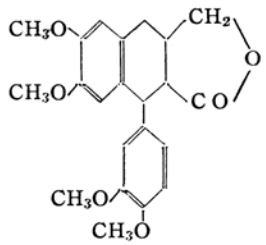
(II)



(III)

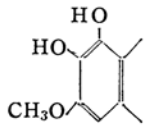


(IV)

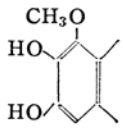


(V)

Amongst these, (III) was excluded through comparison with the absorption of (V)[17] (λ_{max}^{alc} 283 mμ), and similary, (IV) was discarded on grounds of the absorption of 1,2-dimethoxy-4-*n*-amylbenzene[18] (λ_{max}^{alc} 284 mμ), thus leaving (II) as the plausible structure. Accordingly, 1,2,3,4-tetrahydro-5,6,7-trimethoxynaphthalene[19] was synthesized, and it was found that the spectrum was superimposable on that of the pristimerol derivative (Fig. 4). This proved in a conclusive manner that in pristimerol the part structure (VI) or (VII) was



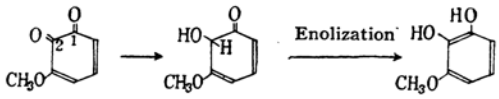
(VI)



(VII)

15) (i) For the present discussion, the chromophoric power of a phenolic hydroxyl and its methyl ether is regarded as being approximately identical. (ii) Alkyl groups attached to benzenoid nuclei produce bathochromic shifts. However, when an oxygenic function is attached to the nucleus, a bathochromic shift is not necessarily the result, e.g., catechol (λ_{max} 278 mμ) and pyrogallol (λ_{max} 267 mμ). Hence, comparisons should be made only amongst compounds which are substituted by oxygenic functions in an analogous manner.

present. In agreement with these *o*-dihydroxybenzene structures, pristimerol gave a green ferric chloride test. The obvious fact that pristimerin should necessarily be the corresponding quinone received confirmation from the results of catalytic hydrogenation, i. e., pristimerin absorbed only one mole of hydrogen, and rapid processing of the colorless solution afforded the expected pristimerol. Formation of pristimerol by sodium borohyride may be presumed to proceed through the following sequences, in which only the

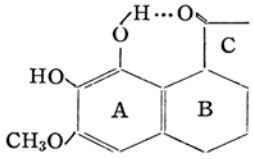


carbonyl group at position 2 is reduced since the conjugated system incorporating the other carbonyl group, $-CO-C=C-C=C-OCH_3$, may be regarded as a carbomethoxy vinylog. Formation of pristimerol with methanolic hydrochloric acid possibly proceeds through an intermolecular oxidation-reduction. (In fact, the yield of pristimerol by this latter method never exceeded 50%.)

The position and nature of the remaining oxygenic function will now be discussed. The oxygenic functions in positions 1 and 2 are hindered, as evidenced from the unreactivity of pristimerin with *o*-phenylenediamine, and recovery of pristimerol upon attempts to obtain a carbonate by treating with phosgene. Furthermore, the carbonyl group apparent in the infrared spectra of the reductive acetate, pristimerol, and its dimethyl ether (Figs. 2b, 2d, 2e) afforded no carbonyl derivatives and the dimethyl ether resisted reduction with sodium borohydride or lithium aluminum hydride. These facts may be satisfied by assuming a rather rigid structure for these functions i. e., by adopting (VI) for pristimerol and placing the carbonyl group in the steri-

16) R.A. Morton and Z. Sawires, *J. Chem. Soc.*, 1052 (1940).
17) G.N. Walker, *J. Am. Chem. Soc.*, 75, 3393 (1953).
18) R. Adams, C.K. Cain, and H. Wolff, ibid., 62, 732 (1940).
19) R.D. Haworth, B.P. Moore, and P.L. Pauson, *J. Chem. Soc.*, 3271 (1949).

cally hindered position of an angular tricyclic system as in (VIII). The bathochromic location



(VIII)

of the ketone band in the infrared spectrum of pristimerol (1705 cm^{-1}) as compared with the reductive acetate (1725 cm^{-1}) and pristimerol dimethyl ether (1720 cm^{-1}) may thus be satisfactorily explained by formation of the seven-membered hydrogen bond. Representation of this ketone group in a five-membered rather than in a six-membered ring C was deduced from the infrared absorption of steroidal[20] and triterpenoid[21] ketones; the fact that the analogously constructed 11-keto in the five-membered ring C of hydrojervine derivatives also absorb in the region of 1725 cm^{-1} [22] is also to be noted. However, possibilities for a six-membered ring C still remains.

The 11-oxygenic function in pristimerin has been formulated in the enol-form rather than in the alternative keto-form for the following spectroscopic reasons: (i) Contrary to the statement of Kulkarni and Shah[7], the infrared spectra of pristimerin revealed a weak but distinctive band at 3370–3380 cm^{-1} (in chloroform and carbon tetrachloride), in spite of the fact that the samples used were repeatedly recrystallized from absolute solvents and thoroughly dried. No hypsochromic shift was observed upon dilution of the solutions, thus indicating formation of an intramolecular hydrogen bond. Furthermore, the fact that the solid spectrum likewise showed an hydroxyl absorption (λ_{max}^{Nujol} 3346 cm^{-1}) excluded the possibilities of this remaining oxygenic group being involved in a keto-enol equilibrium[23]; hence, it had to be assumed that some sort of an hydroxyl was present in pristimerin. (ii) One set of isosbestic points was observed in the ultraviolet spectra of pristimerin measured in alcoholic alkali (Fig. 5). In the light of this observation, it follows that in neutral alcohol (and presumably in other less polar solvents[24]) pristimerin exists solely in the enol-form and that an enol-enolate equilibrium is involved; if pristimerin existed in the keto-form or if a keto-enol equilibrium was involved *two* sets of isosbestic points would have been expected.

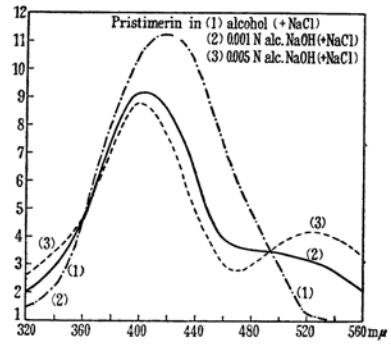


Fig. 5. Isosbestic point; 362 mμ (ε=4800), 495 mμ (ε=3300)

The fact that ozonolysis of pristimerin afforded no volatile carbonyl compound precluded the presence of double bonds within the side-chain. Furthermore, since pristimerol and its dimethyl ether do not absorb hydrogen upon catalytic hydrogenation it seems possible that the double bond is situated in a hindered nuclear position, i. e., either to be tri- or tetra-substituted. Though infrared bands of medium strength were observed around 840 cm^{-1} with pristimerin and derivatives, the infrared evidence alone is as yet not decisive for the presence of a tri-substituted double bond.

As already mentioned by Kulkarni and Shah[7], and by Kamat, et al. the similarity in the source and ultraviolet spectrum of celastrol to those of pristimerin demonstrated a close relationship to exist between the two substances. Though celastrol possesses no methoxyl group, it is methylated by diazomethane[3] to give a product, the analyses of which also agree with $C_{30}H_{40}O_4$ (pristimerin) as well as with the reported $C_{23}H_{32}O_3$. Professor O. Gisvold, Minnesota University, U. S. A., kindly furnished us with a sample of celastrol and its monomethyl ether, and it was found that the latter was indeed identical with pristimerin (melting point, ultraviolet and infrared spectroscopy measure-

20) R.N. Jones, P. Humphries, and K Dobriner, *J. Am. Chem. Soc.*, **71**, 241 (1949).
K. Dobriner, E.R. Katzenellenbogen and R.N. Jones. "Infrared Absorption Spectra of Steroids", Interscience, New York (1953).

21) A. Meyer, O. Jeger, V. Prelog and L. Ruzicka, *Helv. Chim. Acta*, **34**, 747 (1951).

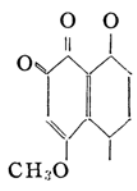
22) O. Wintersteiner, M. Moore and B.M. Iselin, *J. Am. Chem. Soc.*, **76**, 5609 (1954).

23) The infrared spectrum of 1,1-dimethylhexanedione-1,3 in chloroform shows bands arising from both the keto- and enol-form: $\lambda_{max}^{CHCl_3}$ 2630 w, 1702 s, 1724 m, 1605 s (cf., R.S. Rasmussen, D.D. Tunnicliff and R.R. Brattain, *J. Am. Chem. Soc.*, **71**, 1069 (1949); ref. 24); H. Heymann, S.S. Bhatnager and L.F. Fieser, *J. Am. Chem. Soc.*, **76**, 3689 (1954)). However, in nujol the doublet around 1700 cm^{-1} disappeared and only the enolic bands at 2630 (m) and around 1605 cm^{-1} (s) were observed.

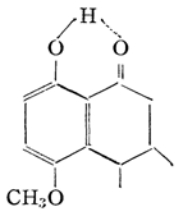
24) In general, the polar ketonic structure is favored in polar solvents, and the chelated enolic form in less polar solvents: cf. B. Eistert and W. Reiss, *Ber.*, **87**, 92 (1954).

ments). Accordingly, the part structure (I') may be assigned to celastrol. The oxidation-reduction potential of pristimerin, as measured by titration, gave the value of ca. +297 mV., which is exceptionally low for *o*-quinones[25]. This is probably due to the combined electron-donating nature of the methoxyl, enol, and alkyl (as ring B) groups.

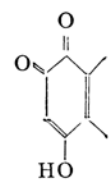
Kulkarni and Shah[7] have forwarded the part structure (IX) for pristimerin. Pristimerol would then be represented by the conjugate-chelated (X), which should now possess the characteristic ultraviolet[25] and infrared[27] absorption of *o*-hydroxyacetophenones, i. e., two, λ_{max}^{alc} at ca. 250 mμ and 330 mμ, as exemplified by 2, 5-dihydroxy-acetophenone (λ_{max}^{alc} 255 mμ, log ε 3.91, and 367 mμ, log ε 3.70), and a strong infrared band around 1635 cm^{-1}, both of which, however, are contrary to facts. Moreover, structure (IX) of pristimerin leads to structure (XI) for celastrol, which would most probably exist in the *p*-quinonoid form (XII) and be methylated to give a *p*-quinone (i. e., pristimerin); the fact that pristimerin



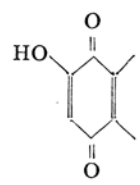
(IX)



(X)



(XI)



(XII)

and celastrol possess identical ultraviolet absorptions excludes the existence of an an $o \rightleftarrows p$ quinone relationship between the two. Kamat, et al.[8], have proposed a carotenoid (conjugated pentaene) structure for pristimerin, which can also be excluded on grounds of ultraviolet absorption since the chromophoric power of the group is insufficient to produce absorption as high as 423 mμ[28].

Experimental

Unless otherwise stated, melting points were determined on a micro hot-stage and are uncorrected. The ultraviolet spectra were measured on Beckman DU and Hitachi spectrophotometers in alcohol. Infrared spectra were measured on Baird, Perkin-Elmer 12C and 21 models, equipped with rock-salt prisms.

Pristimerin.—Pristimerin was recrystallized by adding petroleum ether to a concentrated acetone or benzene solution; orange needles, m.p. 219–220° (dec.). To ascertain that the 3μ infrared band was not caused by water of crystallization, samples of pristimerin were prepared by using freshly prepared absolute solvent pairs of petroleum ether and benzene, and dried over phosphorus pentoxide in vacuo at 100° for fortyeight hours; however, the crystals thus obtained still possessed the same melting point and infrared absorption (Fig. 2a): λ_{max}^{Nujol} 3350 w, 1742 s, 1736 s, 1662 w, 1595 s, 1550 m, 1519 m; $\lambda_{max}^{CCl_4}$ 3380 w, 1740 s, 1655 w, 1607 s, 1514 cm^{-1}. It is considered that the bands around 1740 cm^{-1} are associated with the *o*-quinonoid group; in celastrol, the corresponding band was shifted to 1701 cm^{-1} with a slight shoulder at 1742 cm^{-1}. Ultraviolet spectrum (Fig. 1): λ_{infl} 250–255 mμ (log ε 3.90), λ_{max} 423 mμ (log ε 4.05).

Calcd. for $C_{30}H_{40}O_4$: C, 77.55; H, 8.68; M.W., 464.62.
Found: C, 77.54, 77.84, 78.18, 77.70; H, 8.87, 8.90, 8.72, 8.67; M.W. (Rast), 394.
C–CH_3 (Kuhn-Roth): 8.08% (2.5 groups).
O–CH_3 (Zeisel): 7.69% (1.1 groups).

Attempted Acetylation.—In spite of the several conditions tested, only an amorphous white powder was obtained by regular acetylation (with combinations of acetic anhydride, pyridine, boron trifluoride, sodium acetate, sulfuric acid), repeated purifications by chromatography, and recrystallization from various solvents; m.p. 120–150°C.

Catalytic Hydrogenation of Pristimerin.—In three micro measurements conducted in acetic acid with Adams' catalyst, 1.30, 0.93, and 1.12 moles of hydrogen were absorbed to give a colorless solution, which regained the original orange color when left in contact with air for several hours (cf. Reductive acetate (ii), and pristimerol (iii)).

Reductive Acetate.—(i) A mixture of 150 mg. of pristimerin and 150 mg. of zinc dust was treated with 0.6 cc. of acetic anhydride and one drop of triethylamine, when decolorization took place in five minutes. The mixture was boiled for several minutes, extracted with 1.5 cc. of hot acetic acid, the boiling extract treated with several drops of water and the precipitate recrystallized twice by addition of methanol to a saturated acetone solution; white needless, 90 mg., m.p. 252°, $[\alpha]_D^{22°}$ +54.3° (chloroform). Ultraviolet spectrum (Fig. 1): λ_{max} 278 mμ (log ε 2.90). Infrared spectrum: Fig. 2b. It does not react with carbonyl reagents.

25) K. Wallenfels and W. Möhle, ibid., 86, 926 (1953).
26) R.A. Morton and A.L. Stubbs, *J. Chem. Soc.*, 1347 (1940).
27) H.L. Hegert and E.F. Kurth, *J. Am. Chem. Soc.*, 75, 1622 (1953).
28) W. Oroshnik and A.D. Mebane, ibid., 76, 5719 (1954).

Calcd. for $C_{34}H_{46}O_6$: C, 74.15; H, 8.42.
Found: C, 73.75, 73.96; H, 8.29, 8.10.
$-OCOCH_3$ Content: 1.8 groups.

(ii) The same reductive acetate (m.p. and infrared spectrum) was obtained by hydrogenating 100 mg. of pristmerin in 6 cc. of acetic anhydride over PtO_2 catalyst, adding 1 cc. of pyridine, leaving the solution overnight, removing the catalyst, and treating as in (i).

Ozonolysis of Pristimerin.—Ozone was passed through a solution of 60 mg. of pristimerin in 5 cc. of chloroform until complete decolorization took place, and the ozonide was decomposed by boiling with water. No precipitate was formed in a solution of 2,4-dinitrophenylhydrazine connected to the top of the condenser during the refluxing; steam distillation of the ozonolysis mixture likewise afforded no 2,4-dinitrophenylhydrazone.

Zinc Distillation.—One end of a glass tubing (7×0.6 cm.) was drawn out into a capillary and the root of the capillary was stuffed loosely with some asbestos. The tubing was filled in the following order with zinc-dust, pristimerin+zinc-dust (1: 100 mixture), and zinc-dust, to make layers of 1 cm. (a), 0.5 cm. (b), and 0.5 cm. (c), respectively, and the tube was sealed outside layer c. After first heating (a) until red-hot with a microflame, the tubing was heated from (c) through (b) towards (a) during a period of three to four minutes. During the course of distillation, care should be taken to distil with heat transmitted through the zinc layer and to move the flame slowly towards new portions of the tube containing the yet undecomposed sample. Ninety of these distillations were carried out on a mixture of 600 mg. of pristimerin and 60 g. of zinc-dust. The respective capillary portions containing an oily and crystalline aromatic distillate were cut off, washed with acetone, and the solutions combined. The solvent was evaporated and the ethereal solution of the residue was passed through a column (0.5×10 cm.) of alumina (acid-washed), when a purple-red band remained sticking on the top. The eluate was passed through a column twice more and the ether was evaporated. The dark-yellow residue was submitted to a fractional dissolution using a 1: 2 mixture of acetone and petroleum ether, when a crystalline product was obtained from the fraction which was more soluble in the solvent pair. The other fraction was an orange oil with an aromatic odor and an ultraviolet spectrum resembling naphthalenoid compounds. The crystals were combined and passed through a column once more and finally recrystallized from ethanol, when 3 mg. of slight yellow flakes with a strong purple fluorescence were obtained; m.p. 299–300° (dec.) (in sealed tube, otherwise sublimation). Ultraviolet spectrum (Fig. 3); 232 mμ (log ε 4.32), 257 (4.63), 275 (4.78), 284 (4.90), 297 (shoulder, 4.40), 301 (4.39), 313 (shoulder, 4.15), 327 (4.17), 357 (2.97), 376 (2.87). Infrared spectrum: Fig. 2c.

Calcd. for $C_{25}H_{22}$: C, 93.40; H, 6.60.
Found: C, 93.43; H, 6.87. (analysis carried out on 0.975 mg.)

Pristimerol.—(i) A solution of 50 mg. of pristimerin in 2 cc. of ethanol was decolorized immediately upon addition of a small amount of sodium borohydride. After several minutes, the excess reagent was decomposed with acetic acid, and hot water was added dropwise to the boiling alcoholic solution until the solution became slightly turbid; 48 mg of white needles, m.p. 241° (ethanol-water). Acetylation of pristimerol under a variety of conditions (including procedure for preparation of reductive acetate) gave only an amorphous product. Attempts to obtain a carbonate of this *o*-dihydroxybenzene derivative by reacting with phosgene, or to prepare carbonyl derivatives resulted in recovery of starting material. Addition of *p*-diazobenzenesulfonic acid dissolved in aqueous sodium carbonate to an acetic solution of pristimerol gave rise to a red-orange coloration. Ultraviolet spectrum (Fig. 4): λ_{max} 284 mμ (log ε 3.49). Infrared spectrum: Fig. 2d.

Calcd. for $C_{30}H_{42}O_4$: C, 77.21; H, 9.07.
Found: C, 77.12, 77.08; H, 9.02, 9.22.
$O-CH_3$ (Zeisel): 5.42% (0.81 groups).

(ii) After adding 1.8 cc. of concentrated hydrochloric acid to 75 mg. of pristimerin dissolved in 10 cc. of ethanol, the solution was boiled for twenty minutes, when it was decolorized. After cooling, the solution was poured into 30 cc. of water, and the precipitates were collected, dried, and recrystallized from benzene, 25 mg., m.p. 236°.

Found: C, 77.35, 77.27; H, 9.14, 9.31.

(iii) A solution of 40 mg. of pristimerin in 2 cc. of acetic acid and 2 cc. of ethanol was reduced with PtO_2—H_2, the catalyst filtered rapidly, and the solution was worked up as mentioned in (i) to give 22 mg. of crystals, m.p. 234–5°. The samples prepared by methods (ii) and (iii) were identical with pristimerol, as ascertained by infrared spectra and identity of the di-*p*-nitrobenzoate.

Pristimerol Di-*p*-nitrobenzoate.—A mixture of 40 mg. of pristimenol, 1.5 cc. of pyridine, and 60 mg. of *p*-nitrobenzoyl chloride was heated on the bath for one hour, and poured into water after cooling. The excess reagent was dissolved by addition of sodium bicarbonate, and the precipitate was collected, dried, and recrystallized thrice from acetone, to give 30 mg. of white needles, m.p. 213°.

Calcd. for $C_{44}H_{48}O_{10}N_2$: C, 69.09; H, 6.33; N, 3.66.
Found: C, 69.02, 69.20; H, 6.65, 6.43; N, 3.72.

Pristimerol Dimethyl Ether.—To a solution of 100 mg. of pristimerol in 5 cc. of acetone, there was added 100 mg. of finely pulverized potassium carbonate and 0.1 cc. of freshly distilled dimethyl sulfate. After boiling the mixture for three hours, the same amount of potassium carbonate and dimethyl sulfate were added, and the mixture was boiled for another three hours, and filtered while hot. White crystals (70 mg.) were obtained from the filtrate. Concentration of the filtrate yielded another crop (30 mg.) of crystals; m.p. 209–10° (acetone). The substance did not react with carbonyl reagents and was not reduced with lithium aluminum hydride or sodium borohydride. Attempted enol acetylation (acetic anhydride-

pyridine) also failed. The O—CH_3 determination was unsatisfactory owing to its only slight solubility in the decomposition solvent (Zeisel method). Ultraviolet spectrum: λ_{max} 280 mμ (log ε 3.31). Infrared spectrum: Fig. 2e.

Calcd. for $C_{32}H_{47}O_4$: C, 77.69,: H, 9.37.
Found: C, 77. 28, 77.77; H, 9.20, 9 54.

Isosbestic Point of Pristimerin (Fig. 5).—A stock solution of 3.77 mg. of pristimerin in 100 cc. of alcohol was prepared. For measurement of the spectrum in neutral alcohol, one drop of 1 N sodium chloride solution was added from a hypodermic syringe to exactly 3 cc. of the stock solution in a 1 cm. Beckman cell. For measurement in the alkaline region, a solution of sodium hydroxide dissolved in 1 N sodium chloride was added instead, the alkali concentration of which was adjusted so as to give final alcoholic alkali concentrations of 0.0002 N, 0.001 N, and 0.005 N (the spectra were the same at concentrations above 0.005 N). The range 320—600 mμ was measured within two minutes, so as to avoid the effect of atomspheric carbon dioxide. The ultraviolet region, 210–340 mμ, was also measured but the curves did not cross; in this case, the initial inflection around 250 mμ ($\varepsilon=8.3\times10^3$) in neutral ethanol shifted towards longer wave-length with increase in alkali concentration, and was transformed into a well-defined peak in 0.002 N and higher solutions (e.g., 0.005 N solution: λ_{min} 250 mμ, $\varepsilon=9.2\times10^3$; λ_{max} 270 mμ, $\varepsilon=10.8\times10^3$). Apparently the somewhat weak but broad absorption around 520 mμ is responsible, in part, for the brown-red color of alkaline solutions. Acidification with acetic acid of the alkaline solutions restored the spectra to that of neutral pristimerin: however, if the alkaline solutions were left overnight, decomposition occurred.

Oxidation-Reduction Potential of Pristimerin.—A 0.001 molar alcoholic or dioxane solution (pH 1.5) of pristimerin was titrated with a $TiCl_3$—HCl solution, using saturated calomel and platinum electrodes. An accurate oxidation-reduction curve could not be obtained, owing to precipitation of a quinhydrone, but the value was around +297 mV. Polarography did not give satisfactoryresults.

Summary

Part structure (I) has been presented for pristimerin on grounds of chemical and spectroscopic evidences. Celastrol (tripterine) has been shown to be demetylated pristimerin, hence (I').

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