807. *Pristimerin. Part I. The Nature of the Chromophore.*

By P. K. Grant and A. W. Johnson.

The partial structure (V) is suggested for the wood pigments, pristimerin and celastrol. Structures are also proposed for dihydropristimerin (pristimerol), as well as an oxidation product of pristimerol dimethyl ether and the products obtained from pristimerin by Thiele acetylation and by acid rearrangement. The naphthalenoid systems present in the last two compounds are not identical; both are built up from the original quinonoid ring but by extensions involving different adjacent rings.

Pristimerin is an orange crystalline compound, first isolated from *Pristimera indica* (Wild) (*Hippocratea indica*) and from *P. grahami* of the family Celastraceae, by Bhatnagar and Divekar [1] who claimed that it was active against Gram-positive organisms and the *Viridans* group of streptococci. A closely related compound, celastrol, was isolated from the root bark of *Celastrus scandens* [2,3] and is identical with tripterine, obtained [4] from the powdered

[1] Bhatnagar and Divekar, *J. Sci. Ind. Res., India*, 1951, **10**, *B*, 56.
[2] Gisvold, *J. Amer. Pharm. Assoc.*, (*a*) 1939, **28**, 440; (*b*) 1940, **29**, 12; (*c*) *ibid.*, p. 432; (*d*) 1942, **31**, 529.
[3] Chou and Mei, *Chinese J. Physiol.*, 1936, **10**, 529.
[4] Schechter and Haller, *J. Amer. Chem. Soc.*, 1942, **64**, 182.

roots of the thunder-god vine, *Tripterygium wilfordii*, Hook (Celastraceae). The structures of these two compounds, pristimerin and celastrol, are closely related, and we have confirmed that methylation of celastrol with diazomethane gave the corresponding methyl ether (acid OH ⟶ OMe) which is identical with pristimerin.[5,6] We have isolated pristimerin from the outer root barks of *Celastrus dispermus* and *Denhamia pittosporoides* (Celastraceae) kindly obtained for us by Professor Sir Alexander Todd and members of C.S.I.R.O., Melbourne. Counter-current distribution was used for the purification of the pigment; chromatography was largely unsuccessful because of " lake " formation between the pigments and the adsorbent.

Repeated analyses of pristimerin have given results which correspond to the molecular formula $C_{30}H_{40}O_4$ (and therefore $C_{29}H_{38}O_4$ for celastrol) in agreement with the latest views of Kamat *et al.*[7] and Nakanishi *et al.*[6,8] The molecule contains one *O*-methyl group, at least two *C*-methyl groups, and one active hydrogen atom. Shah, Kulkarni, and Thakore[9] favour a formula $C_{28}H_{36}O_4$ for pristimerin and Gisvold[2] preferred $C_{22}H_{30}O_3$ for celastrol.

Most of the chemical work so far reported for these substances has dealt with the nature of the chromophores, and all previous workers agree that both pristimerin and celastrol are *o*-quinones containing a hydroxyl group which is hydrogen-bonded with one of the quinone carbonyl groups. Such a structure is stated to be compatible with the results of preliminary *X*-ray studies.[10] The oxygenated-carotenoid type of structure advanced by Kamat, Fernandes, and Bhatnagar[7] can be discounted on many grounds, especially spectral characteristics and quantitative hydrogenation (see below).

Three variants of the *peri*-hydroxy-*o*-quinonoid chromophore have been suggested: (I)[2c,2d,4,11], (II)[9], and (III)[6,8]; the underlying evidence is (i) the hydrogenation of pristimerin to a dihydro-derivative, pristimerol, which is a dihydric phenol (diacetate, di-*p*-nitrobenzoate, dimethyl ether) and re-forms pristimerin after aerial oxidation, (ii) the decolorisation of pristimerin by solutions of sodium hydrogen sulphite, (iii) the formation of a boroacetate (Dimroth test),[12] and (iv) a resemblance between the shape of the ultraviolet and the visible absorption curve (Fig. 1) and those of typical *o*-quinones. We have confirmed that pristimerin gives an intense olive-green ferric reaction. It is insoluble in aqueous sodium hydroxide but in alcoholic solution gives a strong crimson colour which fades rapidly on dilution. Pristimerin is insoluble in aqueous acids but a deep red colour develops when it is dissolved in concentrated sulphuric acid. It gives a colourless adduct with an alcoholic solution of sodium hydrogen sulphite but fails to give a positive Craven reaction[13] or colour tests with indole or ethylenediamine[14] for benzoquinones or α- or β-naphthaquinones.

Pristimerol, $C_{30}H_{42}O_4$ (1 OMe; 2 OH), and its dimethyl ether, $C_{32}H_{46}O_4$ (3 OMe) (formed by reductive methylation of pristimerin), each show a single band in the ultraviolet absorption spectrum, max. at 284 mμ (log ε 3·49) and 281·5 mμ (log ε 3·33) respectively. These spectra are characteristic of monocyclic phenols or their ethers rather than naphthols, and accordingly structure (I) can be excluded. Likewise structure (II), which should give rise to a dihydroxymethoxyacetophenone on reduction, can be discounted in the light of the spectrum of *o*-hydroxyacetophenone[15] [max. at 327 and 251 mμ (log ε 3·50 and 3·97) in EtOH]. Many features of the spectra of pristimerin cannot be correlated with the Japanese structure (III). Thus, although the shape of the ultraviolet and visible

[5] Kulkarni and Shah, *Nature*, 1954, **173**, 1237.
[6] Nakanishi, Kakisawa, and Hirata, *J. Amer. Chem. Soc.*, 1955, **77**, 3169, 6729.
[7] Kamat, Fernandes, and Bhatnagar, *J. Sci. Ind. Res., India*, 1955, **14**, *C*, 1.
[8] Nakanishi, Kakisawa, and Hirata, *Bull. Chem. Soc. Japan*, 1956, **29**, 7.
[9] Shah, Kulkarni, and Thakore, *J.*, 1955, 2515.
[10] Carlisle and Ehrenberg, *Acta Cryst.*, 1956, **9**, 823.
[11] Fieser and Jones, *J. Amer. Pharm. Assoc.*, 1942, **31**, 315.
[12] Dimroth and Faust, *Ber.*, 1921, **54**, 3020.
[13] Craven, *J.*, 1931, 1605.
[14] Karius and Mapstone, *Chem. and Ind.*, 1956, 266.
[15] Morton and Stubbs, *J.*, 1940, 1351.

spectrum resembles that of a substituted *o*-benzoquinone, the intensity of the visible band at 420—430 mμ is greater than those of the quinones [16] by a factor of nearly ten. Moreover, the *o*-quinonoid system (III), which contains one non-bonded quinonoid-carbonyl group, would be expected to show absorption in the region of 1664 cm.$^{-1}$,[17] but in fact pristimerin in solution shows strong bands at 1720 and at 1586 cm.$^{-1}$ and no intermediate bands are present. In celastrol, the corresponding bands are at 1712 and 1595 cm.$^{-1}$. The difficulty of reconciling the position of these bands with structure (III) was recognised by Nakanishi *et al.* who admitted that the " bands in the 6 μ region are as yet not completely

FIG. 1. *Absorption spectra of (A) pristimerin and (B) celastrol in* 95% EtOH.

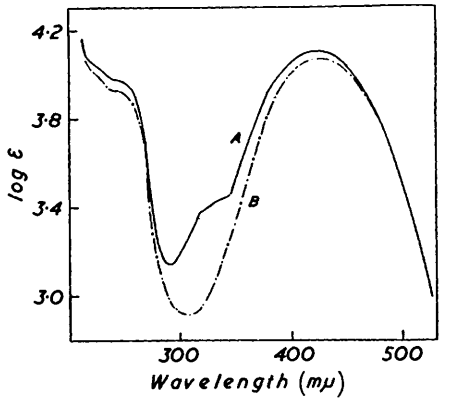
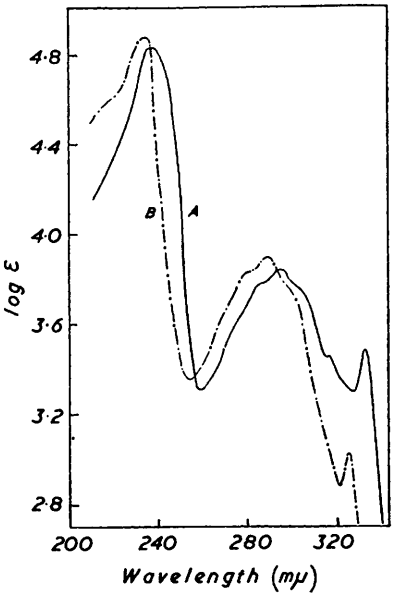
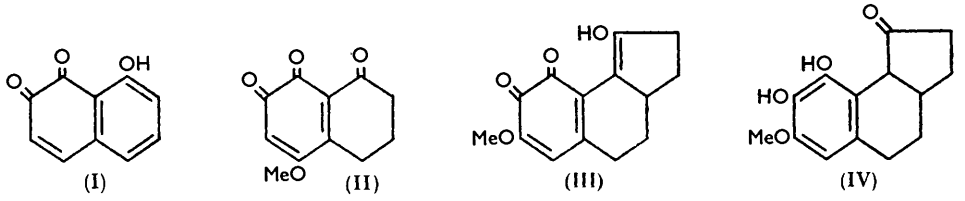


FIG. 2. *Absorption spectra of (A) the acid-rearrangement product and (B) the Thiele acetylation product of pristimerin, in* 95% EtOH.



interpretable." Moreover, pristimerol, which contains an unreactive carbonyl group, was formulated as (IV) by these authors so that the same oxygen function was represented as an enol in pristimerin and a ketone in pristimerol and the band at 1720 cm.$^{-1}$ was associated with the quinonoid-carbonyl group in pristimerin whereas another at 1705 cm.$^{-1}$ in pristimerol or 1724 cm.$^{-1}$ in pristimerol dimethyl ether was attributed to the inert ketone.

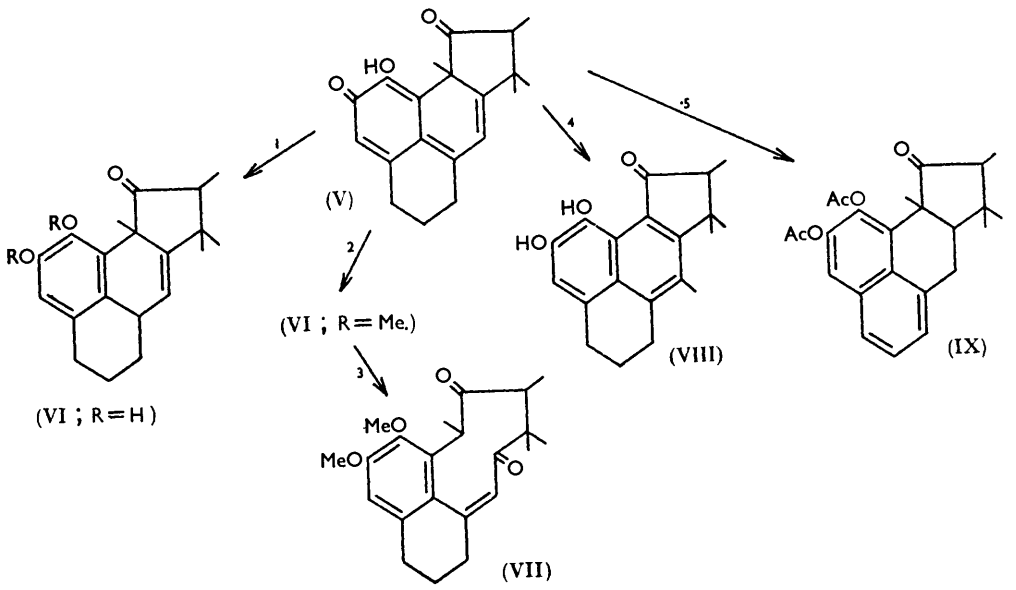


It is probable that these bands at *ca.* 1720 cm.$^{-1}$ are all associated with the inert carbonyl group and that in pristimerol it is hydrogen-bonded with one of the hydroxyl groups. In accordance with this view, pristimerin shows a weak band at 3320 cm.$^{-1}$ (bonded OH) in

[16] Smith, Irwin, and Ungnade, *J. Amer. Chem. Soc.*, 1939, **61**, 2424; Mason, *ibid.*, 1948, **70**, 138; Nakagura and Kuboyana, *ibid.*, 1954, **76**, 1004.
[17] Ötting and Staiger, *Chem. Ber.*, 1955, **88**, 828.

the 3 μ region of the spectrum and pristimerol shows two bands at 3350 (bonded OH) and 3580 cm.$^{-1}$ (OH).

The interpretation of the spectra has led us to abandon the earlier quinonoid structures for pristimerin and in the light of the properties of the derived compounds we propose (V) as the structure of the pristimerin chromophore. The more important reactions of pristimerin are summarised below.



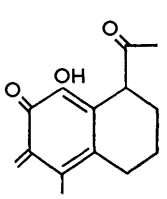
Reagents: 1, H_2; 2, H_2–Me_2SO_4; 3, $KMnO_4$; 4, H_2SO_4; 5, H_2SO_4–Ac_2O.

The proposed structure for pristimerin (V) is a hydroxy-substituted methylenequinone, and pristimerol is therefore represented as a catechol. This is in accord with the observed ultraviolet absorption spectrum of pristimerol, the single absorption maximum rather than a doublet [18] being attributed to a high degree of substitution in the ring. The ultraviolet absorption characteristics of substituted catechols and their methyl ethers are not sufficiently specific to indicate a 5 : 6 : 7 : 8-tetrahydro-1 : 2 : 3-trimethoxynaphthalene structure for pristimerol dimethyl ether as claimed, on the basis of this property only by the Japanese authors.[6,8] Evidence to be presented below makes it clear that the methoxy-group of pristimerin cannot be a substituent of the quinonoid ring. There is other evidence to support the view that pristimerol is a substituted catechol. It gives a green ferric reaction and a positive Asahina reaction,[19] and in alkaline solution it darkens rapidly as oxidation proceeds, to re-form pristimerin; the presence of borate retards pristimerol in chromatography on borate-treated paper [20] and the infrared spectrum of the diacetate shows a single band at 1773 cm.$^{-1}$ (carbonyl of aromatic acetate) as well as the band at 1736 cm.$^{-1}$ (inert ketone group).

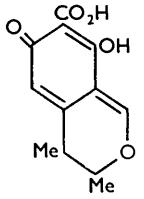
A negative Gibbs test [21] suggests that both positions *para* to the hydroxyl groups are substituted, as in (VI), but the fact that pristimerol couples with *p*-sulphobenzenediazonium salts [8] indicates that the sixth position in the catechol ring is unsubstituted. This eliminates an *o*-methylenequinone structure such as (X) for pristimerin, which would then give a fully substituted catechol on reduction.

[18] Adams, Cain, and Wolff, *J. Amer. Chem. Soc.*, 1940, **62**, 732.
[19] Asahina, Asano, and Ueno, *Bull. Chem. Soc. Japan*, 1942, **17**, 105.
[20] Swain, *Biochem. J.*, 1953, **53**, 200.
[21] King, King, and Manning, *J.*, 1957, 563.

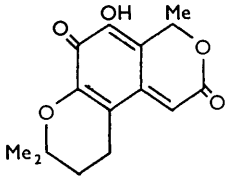
The maximum in the absorption spectrum of pristimerin in the visible region [420—425 mμ (log ε 4·1)] is at an appreciably higher wavelength than those of simple *p*-methylenequinones, *e.g.*, citrinin [22] (XI) [333 mμ (log ε 3·92)] and fuscin [23] (XII) [355 mμ (log ε 4·44)], and the chromophore has therefore been extended by an additional double bond in pristimerin (V). For comparison, hæmatein (XIII) absorbs at 430 mμ (log ε 4·60).[24] If pristimerin is indeed pentacyclic as is suggested by the isolation of an alkylpicene after zinc dust distillation [8] then, when oxygen functions are ascribed to two



(X)



(XI)

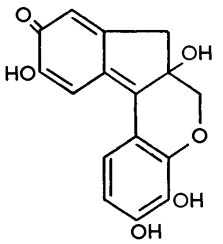


(XII)

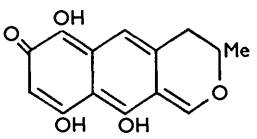
carbonyl groups and one hydroxyl and one methoxyl group, the molecule must contain four double bonds on the basis of the formula $C_{30}H_{40}O_4$.

The dihydro-compound, pristimerol, is formulated as (VI; R = H) in which the additional double bond is not conjugated with the benzenoid ring in accordance with the ultraviolet absorption spectra, which are distinct from those of substituted styrenes [25] and of pristimerol and its derivatives. Pristimerol dimethyl ether gives a positive test for unsaturation with tetranitromethane.

Most of the known methylenequinones contain an oxygen function at the end of the conjugated system remote from the diunsaturated carbonyl group, *e.g.*, fuscin (XII)



(XIII)



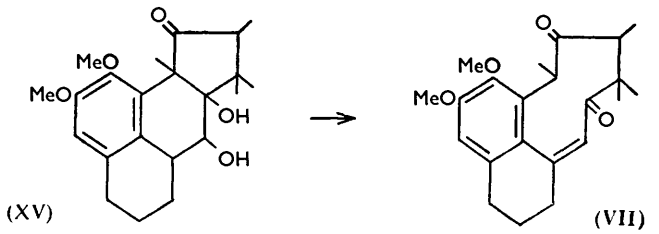
(XIV)

contains another carbonyl group, and citrinin (XI), purpurogenone [26] (XIV), hæmatein [27] (XIII) and the closely related brazilein contain a cyclic ethereal oxygen atom. This feature, which isolates the chromophore from labile hydrogen atoms and prevents a tautomeric rearrangement of the colouring matter to a phenol, is not contained in the proposed chromophore (V) of pristimerin. It has been assumed therefore that neither of the carbon atoms terminating the chromophoric system bears a hydrogen atom, which is in accord with the observation that pristimerol shows no tendency to oxidise to a naphthalene derivative. The stability gained by structure (V) by hydrogen bonding between the diunsaturated carbonyl and the hydroxyl group as well as from the incorporation of the additional double bond into the conjugated system presumably accounts for re-oxidation of pristimerol to (V) rather than to the corresponding *o*-quinone.

The infrared spectrum of pristimerin gives evidence of the bonded hydroxyl group

[22] Brown, Robertson, Whalley and Cartwright, *J.*, 1949, 867.
[23] Barton and Hendrickson, *J.*, 1956, 1028.
[24] Cooke and Segal, *Austral. J. Chem.*, 1955, **8**, 107.
[25] Braude, Jones, and Stern, *J.*, 1947, 1087.
[26] Roberts, *Chem. Soc. Special Publ.*, 1956, No. 5, 36.
[27] Engels, Perkin, and Robinson, *J.*, 1908, **93**, 1115.

(weak band at 3320 cm.$^{-1}$) together with intense bands at 1586 and at 1720 cm.$^{-1}$ (in chloroform solution). The last band is retained in pristimerol and its derivatives and is ascribed to the inert carbonyl group, and hence the 1586 cm.$^{-1}$ band, although very low for a carbonyl grouping, must represent the diunsaturated carbonyl group of the chromophore. For comparison, the similar group of fuscin [23] absorbs at 1638 cm.$^{-1}$, citrinin at 1633 cm.$^{-1}$, purpurogenone [26] at 1618 cm.$^{-1}$, tropolone [28] at 1615 cm.$^{-1}$, certain substituted tropolones,[29] *e.g.*, puberulic acid and nootkatin, at 1595 cm.$^{-1}$, and hæmatein at 1595 cm.$^{-1}$. Such a low carbonyl frequency is often indicative of an enolisable β-diketone system but this could not give rise to a catechol after reduction. The low frequency of the carbonyl absorption of pristimerin is attributed partly to the fact that it is incorporated in a large rigid structure and partly to the strong hydrogen-bonding which exists between this carbonyl and the hydroxyl group. This is shown by the formation of a series of chelated metallic derivatives, *e.g.*, with copper, nickel, and aluminium, from pristimerin. The copper compound, which is soluble in organic solvents, shows a new absorption band in the visible spectrum near 530 mμ and in the infrared region it no longer shows strong absorption at 1586 cm.$^{-1}$ but instead there appears a new intense band at 1532 cm.$^{-1}$ and the absorption at 1723 cm.$^{-1}$ associated with the inert carbonyl group is unaffected. In this connection, in view of the position of absorption of the corresponding 11-keto-group in the steroid series [30] (1710—1716 cm.$^{-1}$ in carbon disulphide; 1703 cm.$^{-1}$ in chloroform) and that of the 11-keto-group (contained in a five-membered ring) in certain derivatives of jervine [31] (*ca.* 1727 cm.$^{-1}$ instead of 1745 cm.$^{-1}$ for the *cyclo*pentanone-carbonyl group), we agree with the Japanese authors [6, 8] that the inert ketone group of pristimerin is probably contained in a five-membered ring. Further evidence bearing on this point is adduced below.



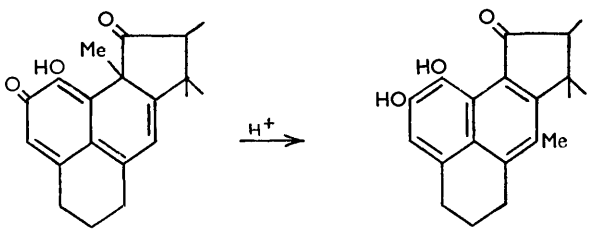
Evidence to support the existence of a non-conjugated double bond in the position shown in pristimerol and its derivatives (VI) has been obtained from the oxidation of the dimethyl ether (VI; R = Me) with potassium permanganate in acetone. Pristimerol dimethyl ether, $C_{32}H_{46}O_4$, contains three methoxyl groups and the inert carbonyl group (infrared band at 1724 cm.$^{-1}$). From the oxidation there was obtained a colourless crystalline neutral product, $C_{32}H_{44}O_5$, which showed maxima at 301 and 249 mμ (log ε 4·01 and 4·16 respectively) and still contained the three methoxyl groups and the inert carbonyl group (band at 1723 cm.$^{-1}$) as well as a new carbonyl group (infrared band at 1643 cm.$^{-1}$). The oxidation product formed a 2 : 4-dinitrophenylhydrazone by the condensation with the new carbonyl group, for the infrared spectrum of the derivative still showed a strong band (1732 cm.$^{-1}$) corresponding to the inert ketone although the 1643 cm.$^{-1}$ band had now disappeared. The ultraviolet and visible absorption spectrum of the 2 : 4-dinitrophenylhydrazone showed bands at 404—405 (log ε 4·48) and 258—261 (log ε 4·29), which suggested that the active carbonyl group of the parent ketone was conjugated to a benzene ring through two double bonds.[32] However as the effect of a *p*-methoxy-group is

[28] Koch, *J.*, 1951, 512.
[29] Aulin-Erdtman and Theorell, *Acta Chem. Scand.*, 1950, **4**, 1490.
[30] Jones, Humphries, and Dobriner, *J. Amer. Chem. Soc.*, 1949, **71**, 241; Cole and Thornton, *J.*, 1956, 1007.
[31] Wintersteiner, Moore, and Iselin, *J. Amer. Chem. Soc.*, 1954, **76**, 5609.
[32] Dr. C. J. Timmons, personal communication.

known [33] to be roughly equivalent to one conjugated double bond the chromophore can be modified to *p*-MeO·C_6H_4·C:C·CO–, a view which is substantiated by the ultraviolet absorption of the ketone itself. For comparison, *p*-methoxybenzylideneacetone [33] shows maxima at 318 and 232 mμ (log ε 4·39 and 4·02). The position of absorption of the carbonyl group of benzylideneacetone [34] is at 1666 cm.$^{-1}$ but this would be lowered in presence of the *p*-methoxy-group.[35]

It is suggested that this oxidation involves hydroxylation of the isolated double bond to yield the diol (XV), and the oxidation product is represented, provisionally, as (VII); its failure to absorb hydrogen in the presence of Adams catalyst is attributed to steric hindrance. One of the hydroxyl groups so introduced now forms part of an aldol system involving the inert ketone group; thus, by the operation of a reverse aldol reaction a new carbonyl group can be obtained, which, after a dehydration involving the second hydroxyl group, is conjugated with the aromatic ring through a double bond.

One of the characteristic properties of the methylenequinones is the ability to rearrange in the presence of acids, bases, and certain other reagents, to yield substituted phenols. When pristimerin was heated with dilute sulphuric acid it gave a colourless dihydric phenol, $C_{30}H_{40}O_4$, which contained one methoxyl group and gave a blue-green ferric reaction, but failed to react with 2 : 4-dinitrophenylhydrazine. The infrared spectrum showed bands at 3525 (OH), 3260 (bonded OH), and 1684 cm.$^{-1}$ (inert C:O) and this compound is the only derivative of pristimerin encountered where the frequency associated with the inert carbonyl group is appreciably changed from 1720 cm.$^{-1}$ apart from intramolecular hydrogen-bonding effects which reduce the frequency to 1710—1715 cm.$^{-1}$ (in $CHCl_3$). The band at 1684 cm.$^{-1}$ suggested that the carbonyl group was conjugated with a double bond or with an aromatic ring and this view was substantiated by the ultraviolet absorption spectrum [max. at 333, 317, 294, and 238 mμ (log ε 3·49, 3·46, 3·85, and 4·73 respectively)] (Fig. 2), which supported the view that the ketone was conjugated with a naphthalene ring.[33,36] The ferric reaction and the retardation of the compound in the presence of borate during chromatography on paper suggested that the compound was an *o*-dihydroxynaphthalene. Like pristimerol the product coupled with diazotised sulphanilic acid, indicating that there was an unsubstituted position in the phenolic ring. It was also of interest that, whereas an alkaline solution of pristimerol darkened rapidly in air, the acid rearrangement product, containing the 1 : 2-naphthalenediol rather than the catechol system, was stable in alkaline solution. The formation of the naphthalene ring in a position such that it is conjugated with the inert carbonyl group, must involve the migration of the angular methoxyl or methyl group. In the former case, the rearrangement would involve an anionotropic shift and in the latter, a double Wagner–Meerwein shift.

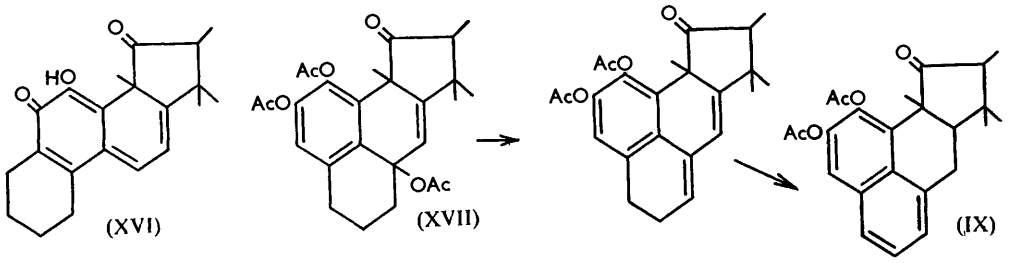


The Japanese claim [8] to have obtained pristimerol by the action of ethanolic hydrochloric acid on pristimerin was based on an intermolecular oxidation–reduction but this result is in need of confirmation. Models of the substance (VIII) show that there is considerable steric hindrance between the two oxygen atoms of the bonded hydroxyl and

[33] Wilds, Beck, Close, Djerassi, Johnson, Johnson, and Shunk, *J. Amer. Chem. Soc.*, 1947, **69**, 1985.
[34] Shigorin, *Doklady Akad. Nauk S.S.S.R.*, 1954, 769; *Chem. Abs.*, 1954, **48**, 11,191.
[35] Soloway and Friess, *J. Amer. Chem. Soc.*, 1951, **73**, 5000.
[36] Daglish, *ibid.*, 1950, **72**, 4859.

the carbonyl group. The strain is diminished when the ketone is part of a five-membered rather than a six-membered ring and this observation supports the spectral evidence quoted earlier which bears on this point.

When pristimerin was treated with sulphuric acid and acetic anhydride under Thiele conditions, a colourless acetyl derivative, $C_{34}H_{44}O_6$, was obtained which contained two aromatic acetate groups. The infrared spectrum showed principal bands at 1776 (aromatic acetate-carbonyl) and 1729 cm.$^{-1}$ (inert ketone) and the ultraviolet absorption [max. at 326, 289, and 233 mμ (log ε 3·04, 3·90, and 4·87 respectively)] (Fig. 2) once again suggested a naphthalene ring,[37] as it is known [38] that the presence of aromatic acetoxy-groups does not affect the shape of the ultraviolet absorption curve of the parent hydrocarbon. However, the frequency of the absorption associated with the inert carbonyl function showed that this group was not in conjugation with the naphthalene ring system, as it had been in the acid rearrangement product (VIII), and, as the original methylenequinone had clearly been converted into the diacetate of a dihydric phenol another ring has been added to the chromophore as in (V). This ring system is preferred to the alternative (XVI) on the grounds that the formulation of pristimerol (VI; R = H) corresponding to (V), as well as the acid rearrangement product (VIII), implies still one unsubstituted position in the catechol ring to allow for the coupling reactions. Several examples have been quoted [39] where the product from a dienone–phenol rearrangement is controlled by the choice of aqueous acid or acetic anhydride as the reaction medium, and in this case the Thiele reaction is visualised as proceeding through the intermediate (XVII), followed by loss of acetic acid and rearrangement of the vinyldihydronaphthalene system to a naphthalene (IX).



It is of interest that the perinaphthenone nucleus has been identified recently [40] in a group of mould pigments, as well as in hæmocorin [24] from the roots of *Haemodorum corymbosum*. The ring system as now represented is not consistent with the formation of an alkylpicene after fusion with zinc,[6, 8] but in view of the drastic experimental conditions and the low yield (3 mg. from 600 mg. of pristimerin) a skeletal rearrangement to a wholly aromatic ring system is a reasonable supposition.

A discussion of other reactions supporting the formulation (V) for the pristimerin chromophore as well as a consideration of the position of the methoxyl group will be presented in a later paper.

Experimental

M. p.s are corrected. Counter-current separations were carried out in an automatic machine with equal 20 c.c. volumes for the two phases. Distribution constants (K) were calculated from the equation $K = r_{max.}/(n - r_{max.})$, where $r_{max.}$ = number of tube containing the highest concentration of solute and n = number of transfers.

[37] Ruzicka, Schinz, and Müller, *Helv. Chim. Acta*, 1944, **27**, 195.
[38] Brockmann and Budde, *Chem. Ber.*, 1953, **86**, 432.
[39] Dreiding, Pummer, and Tomasewski, *J. Amer. Chem. Soc.*, 1953, **75**, 3159; Goodwin and Witkop, *ibid.*, 1957, **79**, 179.
[40] Barton, de Mayo, Morrison, Schaeppi, and Raistrick, *Chem. and Ind.* 1956, 552; Neill and Raistrick, *Biochem. J.*, 1957, **65**, 166.

Isolation of Pristimerin.—The yellow outer bark (100 g.) of *Celastrus dispermus* was removed, ground, and continuously extracted (Soxhlet) with light petroleum (b. p. 60—80°) until no further coloured product was extracted (20 hr.). The solvent was removed from the orange extract under reduced pressure and the crude extract (10 g.) treated with methanol: a colourless solid (0·5 g.) was precipitated, the removal of which greatly increased the efficiency of the subsequent counter-current separation. Repeated recrystallisation of the precipitate from methanol gave colourless crystals, m. p. 197° (Found: C, 79·4; H, 9·85. $C_{20}H_{30}O_2$ requires C, 79·4; H, 10·0%). The product was probably a *diterpene*.

Chromatography of a benzene solution of the crude pigment extract on alumina, magnesium carbonate, or magnesium oxide was unsuccessful.

Preliminary determination of distribution coefficients on the crude extract showed that a *cyclo*hexane–85% aqueous methanol system gave an equal colour distribution but the system used in the actual isolation, *cyclo*hexane–95% aqueous methanol, gave a more equal weight distribution (K 0·95). To suppress emulsification and increase the settling rate, sodium chloride (1 g./l. of lower phase) was added. In a counter-current distribution of the extract (4 g.) contained in three tubes, 98 transfers were carried out. The contents of each tube were evaporated to dryness under reduced pressure and the organic materials separated from the sodium chloride by ether. The ethereal extracts were evaporated to dryness and weighed and a graph of weight *versus* tube number was constructed. Peaks appeared at tube numbers 3, 6—8, 39—42, 69—71, and 87—92. The contents of tubes 65—76 inclusive crystallised to give a further quantity (600 mg.) of the diterpene, m. p. 197°. The contents of tubes 31—48 inclusive (K 0·7) also crystallised, and repeated recrystallisation from ether–light petroleum (b. p. 60—80°) gave orange needles (150 mg., 0·37%) of pristimerin, m. p. 219—220° (Found: C, 77·5, 77·6, 77·6, 77·2; H, 8·5, 8·9, 8·45, 8·8; OMe, 8·5, 8·55; *C*-Me, 6·45; active H, 0·13, 0·38. Calc. for $C_{30}H_{40}O_4$: C, 77·55; H, 8·7; 1OMe, 6·7; 2*C*-Me, 7·3; 1 active H, 0·21%). Light absorption: $\lambda_{max.}$ 420—425 mμ, log ε 4·10; $\lambda_{infl.}$ 335—340 and 251—253 mμ, log ε 3·43, 3·95; $\lambda_{min.}$ 291—293 mμ, log ε 3·13 (Fig. 1). Infrared spectrum (Nujol): max. at 3320 w, 1724 s (1721 in $CHCl_3$), 1654 w, 1586 s, 1547, 1517, 1300 s, 1243 w 1218, 1204, 1185, 1152, 1140, 1082 s, 1030, 867, 860, 848, and 770 w cm.$^{-1}$.

Metal Chelates of Pristimerin.—A solution of pristimerin (50 mg.) in ether (25 c.c.) was shaken with a large excess of saturated aqueous copper acetate for 2 hr. Evaporation of the ethereal layer gave the deep green copper chelate compound of pristimerin (55 mg.). Infrared absorption (Nujol): 1723 s, 1602 w, 1581 w, 1532 s, 1323 w, 1289 s, 1240 s, 1220, 1205, 1139, 1087, and 817 cm.$^{-1}$.

Conversion of Celastrol into Pristimerin.[5, 6]—Treatment of celastrol with an excess of ethereal diazomethane and recrystallisation from ether gave orange needles, m. p., alone and mixed with pristimerin, 219°.

Molecular-weight Determination.—In a microhydrogenation apparatus, pristimerin (29·9 mg.) in dioxan (20 c.c.) in the presence of reduced Adams catalyst (10 mg.) absorbed 1·67 c.c. of hydrogen at 19·5°/766 mm., equivalent to M 426 for pristimerin (1 mol. of hydrogen). A second determination gave M 440. Aerial oxidation converted the reduced compound (pristimerol) into pristimerin (m. p. and mixed m. p. 219°) but there was also obtained a small quantity (*ca.* 2 mg.) of unidentified colourless material.

Pristimerol (*Dihydropristimerin*).—Potassium borohydride was added to a solution of pristimerin (70 mg.) in ethanol (2 c.c.). When the colour of the solution had been discharged, 50% acetic acid was added to destroy the excess of potassium borohydride. Sufficient warm water was added to the boiling ethanolic solution to produce a slight turbidity and, on cooling, pristimerol crystallised. Recrystallisation from light petroleum (b. p. 40—60°) or aqueous ethanol gave colourless needles (40 mg.) of pristimerol, m. p. 226—227° (lit.,[8] 234—235°, 241°) (Found: C, 77·4, 77·15; H, 9·3, 9·25. Calc for $C_{30}H_{22}O_4$: C, 77·21; H, 9·1%), $\lambda_{max.}$ 284 mμ (log ε 3·45), $\lambda_{min.}$ 251—253 mμ (log ε 2·62). Infrared absorption (Nujol): 3540, 3340 s, 1696 s (1720 in $CHCl_3$), 1620, 1508, 1341, 1286 s, 1220 s, 1170, 1112, 1086, 1035, 1017, 998, 879, and 854 cm.$^{-1}$. Paper chromatography with the butan-1-ol–water system: (i) on Whatman No. 1 paper, R_F 0·88; (ii) on Whatman No. 1 borate-buffered paper, R_F 0·73. Pristimerol was detected on the paper by a tetrazotised benzidine spray.

Reductive Acetylation of Pristimerin (*Diacetyldihydropristimerin : Diacetylpristimerol*).—Pristimerin (200 mg.), zinc dust (700 mg.), and anhydrous sodium acetate (35 mg.) were suspended in acetic anhydride (5 c.c.) and glacial acetic acid (1 c.c.). The mixture was gently

heated under reflux for ¼ hr., during which the colour was discharged. The solution was separated from the excess of zinc, and the filtrate poured into ice-water (150 c.c.). The white precipitate was separated and after repeated recrystallisation from chloroform–methanol formed colourless needles (180 mg.) of diacetyldihydropristimerin, m. p. 252° (lit.,[8] 252°) (Found: C, 73·9, 73·7; H, 8·0, 8·0; Ac, 15·0, 15·6; OMe, 7·85. Calc. for $C_{34}H_{46}O_6$: C, 74·15; H, 8·4; 2Ac, 15·6; 1OMe, 5·63%), $\lambda_{infl.}$ 274—276 and 264—268 mμ (log ε 2·67 and 2·78). Infrared absorption (Nujol): 1773 s 1736 s (1723 in $CHCl_3$), 1600, 1299 s, 1239 s, 1220 s, 1186, 1156, 1091, 1053, 1042, 996, 957 w, 904, 873, 859, 837 w, 772, and 675 cm.$^{-1}$.

Deacetylation of Diacetyldihydropristimerin.—The reduced acetyl derivative (100 mg.) was heated under reflux with 1% methanolic sodium hydroxide (25 c.c.) for ½ hr. The mixture was diluted with water, and the methanol removed under vacuum. The residue from an ethereal extract of the resulting precipitate failed to crystallise after removal of the solvent. A 55-transfer counter-current distribution, with *cyclo*hexane–95% aqueous methanol (+NaCl, 1 g./l.), gave pristimerin (60 mg.) (in tubes 23—25, K 0·7) which re-formed the same acetate after reductive acetylation (m. p. and mixed m. p. 251—252°). Tubes 0—2 contained a methanol-soluble component (30 mg.) which did not crystallise.

Reductive Methylation of Pristimerin (Dimethyldihydropristimerin, Pristimerol Dimethyl Ether).—A solution of pristimerin (250 mg.) in methanol (150 c.c.) was reduced to pristimerol by hydrogenation in the presence of Adams catalyst (25 mg.). Dimethyl sulphate (1 c.c.) and 30% aqueous sodium hydroxide (1 c.c.) were added at intervals with vigorous shaking in an atmosphere of hydrogen, until there was no coloration on the addition of the alkali. Shaking was continued overnight to ensure complete methylation. The methanol was removed under reduced pressure and 30% aqueous sodium hydroxide was added to destroy the excess of dimethyl sulphate. The precipitated inorganic salts were dissolved by addition of the minimum quantity of water, and the yellow solid (270 mg.) was separated, dissolved in ether, and chromatographed on alumina (grade I). Removal of the ether from the eluate and recrystallisation of the product from chloroform–methanol gave colourless needles (150 mg.) of dihydrodimethylpristimerin, m. p. 214—215° (lit.,[8] 209—210°) (Found: C, 77·7, 77·4; H, 9·4, 9·5; OMe, 20·2. Calc. for $C_{32}H_{46}O_4$: C, 77·7; H, 9·4; 3OMe, 18·6%), $\lambda_{max.}$ 281—282 mμ (log ε 3·33), $\lambda_{min.}$ 258 mμ (log ε 2·90). Infrared absorption (Nujol): 1724 s, 1595, 1305, 1252 w, 1236 s, 1199 s, 1183 w, 1149, 1136, 1112, 1093 s, 1013 w, 976 s, 964, 877 w, 845 s, 837, 806, 799 w, 773, and 725 cm.$^{-1}$.

Oxidation of the Pristimerol Dimethyl Ether.—Finely powdered potassium permanganate was added in small quantities to the reduced methyl ether (1·38 g.) in acetone (100 c.c.) until a faint pink colour persisted even after prolonged shaking. The manganese dioxide sludge was separated, washed free from potassium permanganate with acetone, and extracted with hot water until no precipitate was obtained on acidification of the filtrate. The combined aqueous filtrates were acidified and extracted with ether (3 × 30 c.c.), and the ether extracts washed and evaporated under reduced pressure. The residue (40 mg.), which was acidic, would still not crystallise.

The acetone filtrate and washings were combined and the acetone was removed *in vacuo* at room temperature, leaving yellow crystals (1·45 g.). Recrystallisation from ether–light petroleum (b. p. 60—80°) gave colourless needles of the oxidation *product*, m. p. 217—218° (Found: C, 75·5, 75·3, 75·3; H, 9·1, 8·6, 8·8; OMe, 20·2; *C*-Me 7·7. $C_{32}H_{44}O_5$ requires C, 75·55; H, 8·7; 3OMe, 18·3; 2*C*-Me 6·7%). Light absorption: $\lambda_{max.}$ 301 and 249 mμ (log ε 4·01 and 4·16); $\lambda_{infl.}$ 219—221 mμ (log ε 4·08); $\lambda_{min.}$ 273 and 232 mμ (log ε 3·67 and 4·02). Infrared absorption (Nujol): 1730 s, 1714 s (single band at 1720 in $CHCl_3$), 1643 s, 1580 s, 1327, 1297, 1280, 1265, 1240, 1202, 1150, 1091 s, 1047 w, 1010 w, 985 w, 965 w, 895, 876, 840, and 793 w cm.$^{-1}$. The 2 : 4-*dinitrophenylhydrazone* formed orange needles, m. p. 278—279° (from ethanol) (Found: C, 66·3; H, 7·0; N, 8·4. $C_{38}H_{48}O_8N_4$ requires C, 66·3; H, 7·0; N, 8·1%). Light absorption: (i) $\lambda_{max.}$ 404—405 and 259—261 mμ (log ε 4·48 and 4·29); $\lambda_{min.}$ 334—335 and 242—244 mμ (log ε 3·72 and 4·23); (ii) in N/100-ethanolic NaOH, $\lambda_{max.}$ 495 mμ (log ε 4·38). Infrared absorption (Nujol): 1732 w, 1616 s, 1587 s, 1517, 1328 s, 1273, 1220, 1160 w, 1134, 1107, 1036, 1014, 967 w, and 829 cm.$^{-1}$.

Acid Rearrangement of Pristimerin.—A suspension of pristimerin in 2N-sulphuric acid (60 c.c.) was heated under reflux in nitrogen. After 14 hr. the pristimerin formed a resin on the surface of the reaction mixture. The solution was cooled, the resin crushed to a powder, and the heating continued. This was repeated at intervals until there was no further change in the appearance of the resin (*ca.* 20 hr.). The mixture was then extracted with ether, and the ethereal layer washed and evaporated, leaving a residue (1·05 g.). This was redissolved in the

ether and on slow evaporation of the solvent crystals separated which recrystallised from methanol as colourless needles (60 mg.) of the rearrangement *product*, m. p. 207—208° (Found: C, 77·3, 77·4; H, 9·0, 8·7; OMe, 8·3. $C_{30}H_{40}O_4$ requires C, 77·55; H, 8·7; 1OMe, 6·7%). Light absorption: $\lambda_{max.}$ 333, 317, 294, and 238 mμ (log ε 3·45, 3·46, 3·84, and 4·78 respectively); $\lambda_{infl.}$ 300—304 and 285—289 mμ (log ε 3·76 and 3·77); $\lambda_{min.}$ 324, 316, and 259 mμ (log ε 3·28, 3·45, and 3·31). Infrared absorption (Nujol): 3525 s, 3260 s, 1684 s, 1631, 1525, 1503, 1350, 1307, 1266 s, 1240, 1223, 1179, 1115 w, 1059, 1020, 955 w, 892 s, 827, 809 s, and 768 w cm.$^{-1}$. Paper chromatography, with the butan-1-ol–water system: on Whatman No. 1 paper, R_F 0·82; on Whatman No. 1 borax-buffered paper, R_F 0·72. The acid rearrangement product was detected by means of a tetrazotised benzidine spray.

The residue from the mother-liquors of the acid-rearrangement product was purified by counter-current distribution, with *cyclo*hexane–90% aqueous methanol (+NaCl, 1 g./l.), 46 transfers being carried out. The contents of the tubes were worked up as described in the isolation of pristimerin, and the main concentration of material appeared at tube numbers 14—16 and 22—24; both fractions were coloured and were not investigated further. Small amounts of crystalline material were isolated from tubes 0—3 and 26—30 inclusive, the product from the latter tubes being identical with the acid rearrangement product (m. p. alone and mixed, 207°).

Thiele Acetylation of Pristimerin.—Pristimerin (100 mg.) was dissolved in cold acetic anhydride (8 c.c.), and concentrated sulphuric acid (1 drop) was added. The initial red colour faded rapidly, a colourless solution being formed which was poured into ice-water (100 c.c.). The acetylated product was separated and chromatographed in ether on silica (6 g.). Removal of the ether from the eluate gave a solid which was crystallised from ether. Recrystallisation of the product from aqueous methanol gave the Thiele acetylation *product*, m. p. 160—161° (Found: C, 74·4, 74·35; H, 8·1, 8·0; Ac, 17·1. $C_{34}H_{44}O_6$ requires C, 74·4; H, 8·1; 2Ac, 15·7%). Light absorption: $\lambda_{max.}$ 325, 289, and 233 mμ (log ε 3·04, 3·90, and 4·87); $\lambda_{infl.}$ 281—282 mμ (log ε 3·83); $\lambda_{min.}$ 321 and 254 mμ (log ε 2·88, 3·66). Infrared absorption (Nujol): 1776 s, 1729 s, 1607 w, 1510 w, 1313 w, 1247, 1224 s, 1175, 1152 w, 1088 w, 1062 w, 1035 w, 1015 w, 900 s, and 822 s cm.$^{-1}$.

We acknowledge the award of a Travelling Scholarship from the University of New Zealand and a maintenance grant from Professor Sir Alexander Todd (to P. K. G.), and numerous helpful discussions with our colleagues, particularly Professor Todd and Dr. T. J. King.

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[*Received, May 6th*, 1957.]