644. *Infra-red Spectroscopy and Structural Chemistry. Part III.* Gladiolic Acid.*

By John Frederick Grove.

The infra-red spectroscopic methods for the study of keto–lactol tautomerism described in Part I (*J.*, 1951, 877) are applied in the present paper to the mould metabolic product gladiolic acid and its derivatives. The conclusions reached are compared with those obtained from an examination of the ultra-violet absorption data for the same compounds in solution. Whereas gladiolic acid and its dibasic oxidation product, $C_{11}H_{10}O_6$, are present in the lactol form in the solid state, an equilibrium between the lactol and the open-chain form exists in aqueous solution, the pH being the determining factor. The esters of gladiolic acid prepared by Grove (*Biochem. J.*, 1952, **50**, 648) are shown to be *pseudo*-esters; and some points of interest in connection with the correlation of absorption frequency with chemical structure are discussed.

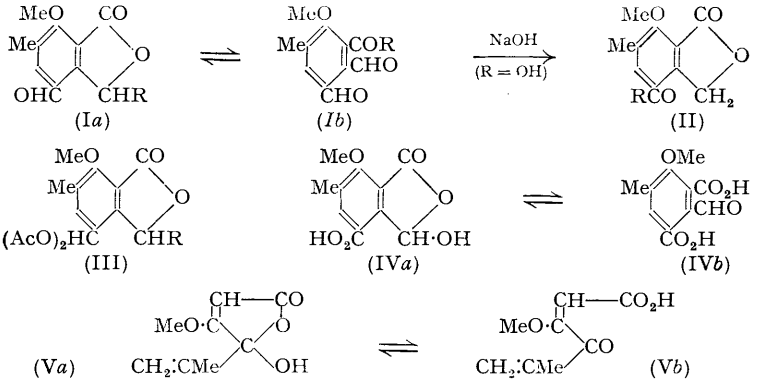
In Part I of this series, Grove and Willis (*J.*, 1951, 877) discussed some of the factors governing the use of infra-red spectroscopy in the study of keto–lactol tautomerism, and showed that the open-chain and the lactol forms of aldehydic and ketonic acids could be recognised by the presence of certain characteristic bands. In addition, it was found that the observed frequencies of the stretching vibrations of the C=O groups could be used to distinguish between normal and *pseudo*-esters of γ-aldehydic and ketonic aicds.

Gladiolic acid, $C_{11}H_{10}O_5$, an antifungal metabolic product of *Penicillium gladioli* Machacek, was first described by Brian, Curtis, Grove, Hemming, and McGowan (*Nature*, 1946, **157**, 697) and was shown to have the structure (I; R = OH) in two papers published simultaneously from these laboratories (Grove, *Biochem. J.*, 1952, **50**, 648) and from the London School of Hygiene (Raistrick and Ross, *ibid.*, p. 635).

Both gladiolic acid and its dibasic oxidation product, $C_{11}H_{10}O_6$ (IV), show keto–lactol tautomerism in solution, under the influence of chemical reagents, and it was of interest to discover the precise structures of these compounds in the solid state. The esters of gladiolic acid described by Grove (1952, *loc. cit.*) were considered to be *pseudo*-esters both

* Part II, Grove, *J.*, 1951, 883.

from their method of preparation and from their chemical properties; their structures are important from the point of view of the correlation of chemical structure with biological activity and a proof of their constitution was desirable.



The present paper deals with the application to gladiolic acid and its derivatives of the infra-red spectroscopic methods described in Part I; in addition, a number of features of interest in connection with the correlation of chemical structure and absorption frequency are discussed.

Experimental

M. p.s are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford.

Infra-red Spectra.—These were obtained by using the modified Hilger D 209 instrument described by Grove and Willis (*loc. cit.*). With the exception of gladiolic acid hemihydrate and penicillic acid hydrate, solid compounds were first dried *in vacuo* over phosphoric oxide. All solid compounds were examined in "Nujol" suspension between rock-salt plates. The suspensions were made by grinding the solids to fine powders in an agate mortar and then stirring the powder with "Nujol." Liquids indicated in Table 1 by an asterisk were examined in a cell of 0·01-mm. thickness. Energy backgrounds were obtained in absence of the sample, and the percentage absorption curves for the region 700—1900 cm.$^{-1}$ plotted in the usual way. Two of the curves are reproduced in Fig. 1. Owing to variations in the thickness of the suspensions used, it is not possible to compare intensities of the absorption bands in the spectrum of one compound with those in another.

Ultra-violet Spectra.—These were obtained by using a Hilger medium quartz spectrograph n conjunction with a "Spekker" ultra-violet spectrophotometer. A hydrogen discharge tube was used as light source, and the concentrations of the solutions were adjusted so that the values of $\log_{10} I_0/I$ between 0·3 and 1·0 were obtained for the absorption peaks with a path length of 0·1 cm.

The data on penicillic acid were obtained with a Unicam S.P. 500 quartz spectrophotometer; this instrument was also used to check some of the results obtained by the older technique. With the Unicam instrument the complete absorption curve was available within 30 minutes of making up the solution, compared with approx. 2 hours in the case of the photographic method.

Materials.—Details of the purification of gladiolic acid and of the preparation of most of the derivatives studied are given by Grove (1952, *loc. cit.*).

Gladiolic acid hemihydrate. Gladiolic acid was allowed to crystallise slowly from a large volume of water, and the colourless prisms of the *hemihydrate*, m. p. 155°, were dried *in vacuo* at room temperature over concentrated sulphuric acid (Found: C, 57·3, 57·2; H, 5·0, 4·9; OMe, 13·8%; equiv., 228. $C_{11}H_{10}O_5,\frac{1}{2}H_2O$ requires C, 57·1; H, 4·8; 1OMe, 13·4%; equiv., 231). The water of crystallisation was readily lost at 100° *in vacuo* over phosphoric oxide and the infra-red spectrum of the dried material was identical in the double-bond stretching region with that of anhydrous gladiolic acid (Found, on a specimen dried for 6 hours as above: C, 59·7; H, 4·4; OMe, 14·6%; equiv., 207. Calc. for $C_{11}H_{10}O_5$: C, 59·45; H, 4·5; 1OMe, 14·0%; equiv., 222).

Esterification of gladiolic acid hemihydrate with methanol under Fischer–Speier conditions gave methyl gladiolate, m. p. and mixed m. p. with an authentic sample, 138° (Found: C, 61·05; H, 5·1; OMe, 26·7. Calc. for $C_{12}H_{12}O_5$; C, 61·0; H, 5·1; 2OMe, 26·2%).

Sodium gladiolate hydrates. Aqueous sodium hydroxide (0·108N; 10·50 ml.) was added to gladiolic acid (0·25 g.) in ethanol (4 ml.), and the resulting solution evaporated to dryness *in vacuo* at room temperature. The solid obtained crystallised from aqueous acetone in colourless needles (dried *in vacuo* at room temperature over sulphuric acid) of *sodium gladiolate trihydrate* (Found: C, 44·7; H, 4·6. $C_{11}H_9O_5Na,3H_2O$ requires C, 44·3; H, 5·0%). Further drying for 4 hours at 100° *in vacuo* over phosphoric oxide gave the *dihydrate*, colourless needles, m. p. 230° (decomp.) (Found: C, 46·8; H, 4·9; OMe, 11·3. $C_{11}H_9O_5Na,2H_2O$ requires C, 47·1; H, 4·7; OMe, 11·1%). When heated slowly, the trihydrate decomposed at the decomposition point of the dihydrate; but, when heated suddenly to 180°, the trihydrate melted, reset, and finally melted (with decomp.) at 230°. The infra-red spectra of the two hydrates were very similar, and differed only in the relative intensities of some of the absorption bands. Both the di- and the tri-hydrate of sodium gladiolate gave an immediate precipitate with Brady's reagent

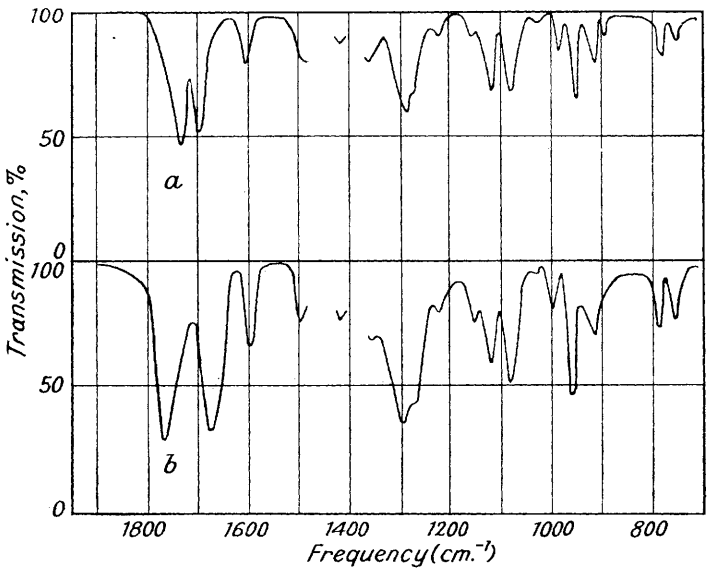


FIG. 1.
a, Gladiolic acid.
b, Gladiolic acid hemihydrate.

in ethanol and after solution in dilute acetic acid gave an intense green colour on the addition of excess of ammonia solution (*d* 0·880). Unlike gladiolic acid, the hydrated sodium salts did not become greenish-brown when suspended in air for a few seconds over ammonia solution (*d* 0·880), and on treatment with ammonia solution (without previous addition of acetic acid) the greenish-brown colour developed only very slowly.

Addition of concentrated hydrochloric acid to an aqueous solution of sodium gladiolate dihydrate gave a colourless precipitate, m. p. and mixed m. p. with gladiolic acid, 158—160°.

When sodium gladiolate dihydrate was dried for 8 hours *in vacuo* over phosphoric oxide at 140° the *monohydrate* was obtained as a yellow solid, m. p. 200—204° (decomp.) (Found: C, 50·5; H, 3·7. $C_{11}H_9O_5Na,H_2O$ requires C, 50·4; H, 4·2%). The material was hygroscopic and it is possible that some decomposition may have taken place during the drastic drying; for these reasons the infra-red spectrum was not examined. The crude monohydrate gave the intense green colour with acetic acid and ammonia solution.

Penicillic acid. The monohydrate, m. p. 64—65°, was obtained by recrystallisation of the crude acid from water (Found, on air-dried material: C, 51·4; H, 6·7. Calc. for $C_8H_{10}O_4,H_2O$: C, 51·1; H, 6·4%). Drying *in vacuo* over phosphoric oxide for 24 hours at room temperature gave the anhydrous acid, m.p. 85° (Found: C, 56·7; H, 5·9. Calc. for $C_8H_{10}O_4$: C, 56·5; H, 5·9%).

Phthalaldehyde. When prepared from *o*-xylene by known methods and crystallised from light petroleum (b. p. 40—60°), this had m. p. 54°.

Results and Discussion

Although the interpretation of the infra-red absorption spectra of organic compounds is generally more reliable when the substances are examined in solution in a non-bonding solvent, Grove and Willis (*loc. cit.*) pointed out the advantages to be obtained from the examination of the spectra of crystalline samples in the study of keto–lactol tautomerism. These are, first, that since carboxylic acids are wholly in the dimeric form in the solid state, the characteristic stretching frequencies of alcoholic –OH and carboxylic acid –OH groups, and of lactone ring and carboxylic acid C=O groups, are more widely separated and hence more readily identified; and, secondly, that the spectra of carboxylic acids are not complicated by the presence of both monomeric and dimeric forms. Nevertheless, some workers have been critical of the use of Nujol "mulls" in an investigation of this kind. Munday (*Nature*, 1949, **163**, 443) claimed that the keto-acid form (V*b*) of penicillic acid was converted into the lactol form (V*a*) by the ordinary procedure of grinding in Nujol and this very naturally raised doubts about the usefulness of the technique in the study of tautomeric systems. However, no analogous effects were observed with any of the acids examined by Grove and Willis (*loc cit.*), and recent work by Ford, Johnson, and Hinman (*J. Amer. Chem. Soc.*, 1950, **72**, 4529), which has been confirmed by the present author, suggests that the phenomenon observed by Munday was actually the conversion of the readily dehydrated penicillic acid (lactol) monohydrate into the anhydrous lactol form (V*a*). Ford *et al.* (*loc. cit.*) pointed out that bond association in crystalline samples affects many of the absorption frequencies and considered it undesirable to base interpretations on the actual positions of bands in the spectra of such samples. Whilst it is true that the C=O frequencies of hydroxylated compounds in the solid state are affected by intermolecular hydrogen bonding [see, for example, the case of *o*-formylbenzoic acid discussed by Grove and Willis (*loc. cit.*) and similar examples in the present paper], these shifts are not large enough to invalidate the interpretation, and the present investigation is confined to the examination of Nujol "mulls."

As in previous papers in this series, discussion of the spectra is limited to the –OH stretching (3—4 μ) and double-bond stretching (5—7 μ) regions; the relevent data are contained in Table 1 and two spectra are reproduced in full over the range 5—14 μ in Fig. 1.

From the data available on phthalaldehyde and benzoic acid (Table 1), the three C=O groups in the keto-form (I*b*; R = OH) of gladiolic acid might be expected to combine to yield a single broad absorption band with maximum at about 1685 cm.$^{-1}$. In fact, gladiolic acid (Fig. 1*a*) shows phthalide ring C=O absorption at 1733 cm.$^{-1}$ and alcoholic OH absorption at 3225 cm.$^{-1}$, close to the values found for *o*-formylbenzoic acid (3-hydroxyphthalide) (Part I), and obviously exists in the lactol form (I*a*; R = OH) in the solid state. The band at 1700 cm.$^{-1}$ in the gladiolic acid spectrum is attributed to the C=O of the formyl substituent; the frequency is slightly higher than that found in benzaldehyde, the difference being due to the resultant electronic effect of the additional substituents. The phthalide ring C=O frequency in *o*-formylbenzoic acid is some 15 cm.$^{-1}$ lower than that found in phthalide itself and this lowering was attributed by Grove and Willis (*loc. cit.*) to intermolecular hydrogen bonding. A similar lowering of the normal C=O and OH frequencies is found with gladiolic acid and suggests intermolecular hydrogen bonding between the phthalide ring C=O groups and lactol OH groups of adjacent molecules. This hypothesis receives considerable support from the results obtained with gladiolic acid hemihydrate in which two molecules of gladiolic acid, instead of being directly associated, are presumably linked through a molecule of water. The spectra of gladiolic acid and its hemihydrate (Fig. 1*b*) are almost identical, except in the C=O and OH stretching regions. The phthalide ring C=O frequency in the hemihydrate appears close to the expected value, 1760 cm.$^{-1}$ (the increase of 10 cm.$^{-1}$ over the normal phthalide figure being due to the substituents attached to the benzene nucleus [cf. deoxygladiolic acid (I*a*; R = H) (Table 1) which absorbs at 1758 cm.$^{-1}$], but the formyl C=O frequency appears at 1680 cm.$^{-1}$, a lowering of 20 cm.$^{-1}$ compared with gladiolic acid. It follows that the molecules of gladiolic acid in the hemihydrate are associated through the water molecule by intermolecular hydrogen bonding involving the formyl groups. The hemihydrate shows two

absorption bands due to OH stretching vibrations in the 3-μ region, at 3495 and 3185 cm.$^{-1}$. It may be presumed that the 3495-cm.$^{-1}$ band arises from the " free " lactol OH groups [*e.g.*, phthalonic acid (3-hydroxyphthalide-3-carboxylic acid), in which dimerisation takes place through the carboxyl group, shows lactol OH absorption at 3470 cm.$^{-1}$, Part I], whilst that at 3185 cm.$^{-1}$ comes from the water molecules involved in hydrogen bonding, although there is no proof of these assignments. Gladiolic acid hemihydrate is readily converted into the anhydrous compound by drying *in vacuo* over phosphoric oxide. In view of Munday's results with penicillic acid it was of interest to see whether the hemihydrate could be converted into anhydrous gladiolic acid by the ordinary procedure of grinding with " Nujol " : the spectra of both gladiolic acid and its hemihydrate were totally unaffected by 10 minutes' grinding with " Nujol " under the conditions normally employed in the preparation of the specimens.

The dibasic acid, $C_{11}H_{10}O_6$ (IV), obtained by oxidation to carboxyl of the formyl group of gladiolic acid which does not take part in keto–lactol tautomerism, shows alcoholic OH absorption at 3390 cm.$^{-1}$ and a phthalide ring C=O frequency at 1760 cm.$^{-1}$, and exists in the lactol form (IV*a*) in the solid state. As might be expected from the similarity

TABLE 1. *Infra-red absorption data for gladiolic acid and its derivatives.*

Compound	Frequency C=O	Frequency OH	Compound	Frequency C=O	Frequency OH
Benzoic acid	1690	2620, 2540	Phthalide	1750	—
Phthalaldehyde	1680	—	*o*-Formylbenzoic acid	1738	3250
Benzaldehyde *	1695	—	*o*-Formylbenzoic acid, *pseudo*-methyl ester	1768	—

* Cf. p. 3346.

Compound	Frequency C=O	Frequency OH
Gladiolic acid (I; R = OH)	1733, 1700	3225
Gladiolic acid hemihydrate	1765, 1680	3495, 3185
Methyl gladiolate (I*a*; R = OMe)	1775, 1705	—
Ethyl gladiolate (I*a*; R = OEt)	1770, 1705	—
Acetylgladiolic acid (3-acetoxy-4-formyl-7-methoxy-6-methylphthalide) (I*a*; R = OAc)	1768, 1700	—
Triacetylgladiolic acid hydrate (3-acetoxy-4-diacetoxymethyl-7-methoxy-6-methylphthalide) (III; R = OAc)	1785, 1765	—
4-Diacetoxymethyl-3 : 7-dimethoxy-6-methylphthalide (III; R = OMe)	1765	—
Deoxygladiolic acid (4-formyl-7-methoxy-6-methylphthalide) (I*a*; R=H)	1758, 1695	—
*iso*Gladiolic acid (7-methoxy-6-methylphthalide-4-carboxylic acid) (II; R = OH)	1755, 1688	2630, 2560
Methyl *iso*gladiolate (methyl 7-methoxy-6-methylphthalide-4-carboxylate) (II; R = OMe)	1766, 1708	—
Oxidation product $C_{11}H_{10}O_6$ (3-hydroxy-7-methoxy-6-methylphthalide-4-carboxylic acid) (IV*a*)	1760, 1688	3390{2605, 2510
Penicillic acid (V)	1742	3280
Penicillic acid hydrate	1723	3400
Sodium gladiolate dihydrate	1580	3425—3125

in chemical structure, the spectrum is almost identical, in the range 5—14 μ, with that of *iso*gladiolic acid (II; R = OH), the compound obtained by rearrangement of gladiolic acid under alkaline conditions. In the dibasic acid, $C_{11}H_{10}O_6$, dimerisation takes place through the free carboxyl groups, and, as in the examples quoted above, the phthalide ring C=O and lactol OH frequencies (1760 and 3390 cm.$^{-1}$, respectively) appear close to the normal values.

Thus, while in the above examples there is a considerable variation in the observed values of the phthalide ring C=O and lactol OH frequencies, the interpretation of the results is not in doubt and gladiolic acid, its hemihydrate, and dibasic oxidation product are all in the lactol form in the solid state.

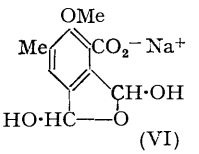
A somewhat similar picture is presented by penicillic acid and its hydrate. The anhydrous acid shows lactone ring C=O absorption at 1742 cm.$^{-1}$ and exists in the lactol form (V*a*); although the single C=O absorption band falls at 1723 cm.$^{-1}$ in the hydrate,

the general pattern of absorption bands in the double-bond stretching region is very similar to that found with the anhydrous material and is quite unlike that predicted for the keto form (V*b*) from data on αβ-unsaturated ketones and carboxylic acids (see Part I). Therefore, although the lactone C=O frequency has been lowered from 1742 to 1723 cm.$^{-1}$ in the hydrate by association with the water molecule, the interpretation of the results is clear and both the anhydrous acid and its hydrate exist in the lactol form (V*a*) in the solid state.

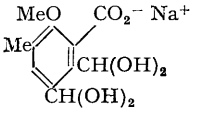
Grove (*loc. cit.*) reported the isolation of methyl and ethyl esters of gladiolic acid and although the chemical evidence, and in particular the formation of non-aldehydic diacetyl derivatives (of the hydrated formyl group), suggested that these compounds were *pseudo*-esters, the structures were not conclusively established. The bands at 1775 and 1770 cm.$^{-1}$ respectively in methyl and ethyl gladiolate may be compared with an absorption of similar frequency in the *pseudo*-methyl ester of *o*-formylbenzoic acid (Part I), and leave no further room for doubt that the compounds are *pseudo*-esters of structure (I*a*; R = OMe and OEt respectively).

Sodium gladiolate could not be obtained anhydrous. Its dihydrate was stable *in vacuo* at 100° over phosphoric oxide, and showed an intense absorption close to 6·3 μ, the maximum falling at 1580 cm.$^{-1}$ with an inflexion at 1612 cm.$^{-1}$ (a band of medium strength close to 1605 cm.$^{-1}$, due to the aromatic ring, is present in all gladiolic acid derivatives examined), and broad unresolved absorption (3425—3125 cm.$^{-1}$) in the OH stretching region. The band at 1580 cm.$^{-1}$ is attributed to the ionised carboxyl group (cf. Thompson, *J.*, 1948, 328) in the keto-form of gladiolic acid, and the absence of bands in the 5·5—6·0 μ region to the fact that sodium gladiolate dihydrate has either the bisdihydroxymethyl structure (VII) or the dihydroxyphthalan structure (VI) with 1 molecule of water of crystallisation.

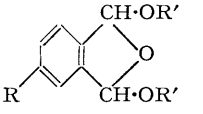
Seekles (*Rec. Trav. chim.*, 1923, **42**, 706) observed that phthalaldehyde formed a monohydrate in water and Weygand, Vogelbach, and Zimmerman (*Ber.*, 1947, **80**, 391) prepared 6-bromo-1 : 3-dimethoxyphthalan (VIII; R = Br, R′ = Me) by the action of methanol on 4-bromophthalaldehyde. It has not been possible to decide between (VI) and (VII),



(VI)



(VII)



(VIII)

the two possible structures for sodium gladiolate dihydrate; a molecule of water was removed from the dihydrate (this was accompanied by some decomposition) by prolonged drying *in vacuo* over phosphoric oxide at 140°, but the resulting material could not be recrystallised and was not investigated further.

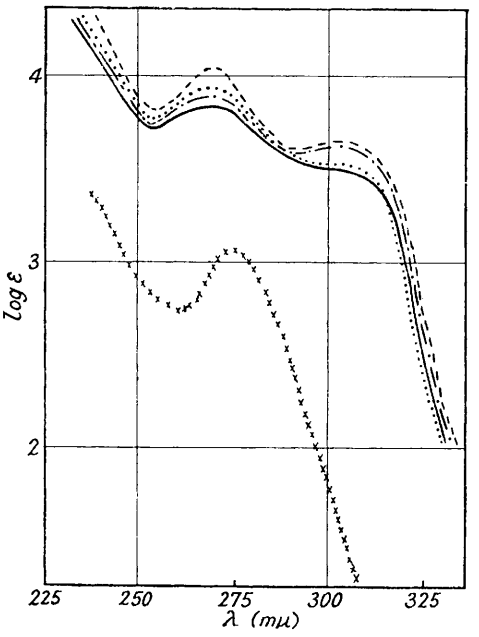
Gladiolic acid and its derivatives provide an interesting example of how structural changes in a series of related compounds can be followed in the infra-red spectra. Thus, acetylation of the lactol hydroxyl group in gladiolic acid to give (I*a*; R = OAc) leads to the disappearance of the OH stretching frequency at 3225 cm.$^{-1}$ and the phthalide ring C=O band moves from the "bonded" position at 1773 cm.$^{-1}$ to 1768 cm.$^{-1}$ (masking the C=O in the acetyl group introduced), whilst the formyl C=O frequency at 1700 cm.$^{-1}$ is unaffected. Acetylation of this group (in its hydrated form) eliminates the band at 1700 cm.$^{-1}$ and a new band appears at 1785 cm.$^{-1}$. A similar sequence can be traced in the formation of the diacetyl derivative of *pseudo*-methyl gladiolate (III; R = OMe). Deoxygladiolic acid (I*a*; R = H) shows phthalide ring and formyl C=O frequencies at 1758 and 1695 cm.$^{-1}$ as expected, and the latter band moves to 1688 cm.$^{-1}$ on oxidation of deoxygladiolic acid to *iso*gladiolic acid (II; R = OH), and to 1708 cm.$^{-1}$ in the normal ester, methyl *iso*gladiolate (II; R = OMe). Thus additional confirmation is provided of the structures allocated to these derivatives.

These structural changes can also be followed in the ultra-violet spectra although the interpretation of the results obtained with gladiolic acid is not straightforward. Fortun-

ately, however, *iso*gladiolic acid (II; R = OH) and the neutral deoxygladiolic acid (I*a*; R = H), in which there is no doubt about the chromophoric systems present, are available to act as models. With their aid the structures of the esters of gladiolic acid can be arrived at independently of the infra-red data and deductions can be made concerning the structure of the gladiolic acid molecule in solution. Without them, the interpretation is ambiguous and uncertain. The ultra-violet absorption data are given in Table 2 and the curves are plotted in Figs. 2 and 3.

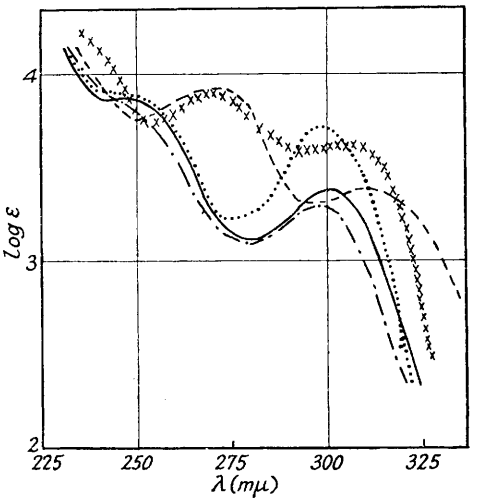
The curves for methyl and ethyl gladiolate and the neutral monoacetyl compound closely follow that for deoxygladiolic acid (I*a*; R = H) with two maxima at 305 and 269 mμ (Fig. 2), and these derivatives clearly have the *pseudo*-structures (I*a*; R = OMe, OEt, and OAc, respectively). Substitution of the formyl C=O chromophore of deoxygladiolic acid

FIG. 2.



— · — · — *Deoxygladiolic acid in ethanol.*
——— *Gladiolic acid in ethanol.*
– – – – – – ,, ,, *water.*
× × × × ,, ,, 0·1N-NaOH.
. . . . . *Ethyl gladiolate in ethanol.*

FIG. 3.



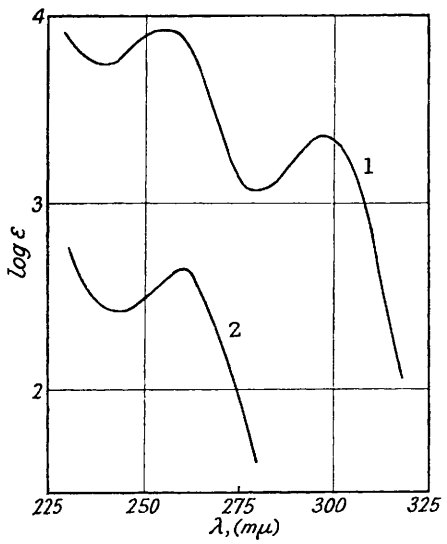
——— *Oxidation product* $C_{11}H_{10}O_6$ *in ethanol.*
— · — · — *Oxidation product* $C_{11}H_{10}O_6$ *in water.*
– – – – – – *Oxidation product* $C_{11}H_{10}O_6$ *in* 0·1N-NaOH.
× × × × *Deoxygladiolic acid in ethanol.*
. . . . . iso*Gladiolic acid in ethanol.*

by a carboxyl substituent (in *iso*gladiolic acid, Fig. 3) gives a different type of curve with a single peak at 298 mμ and an inflexion close to 250 mμ and a similar absorption curve (not reproduced) is given by the triacetyl derivative (III; R = OAc).

When the ultra-violet spectrum of a substance capable of showing keto–lactol tautomerism is examined for solutions in an excess of dilute sodium hydroxide, there is generally a displacement of the absorption maxima to longer wave-lengths compared with the absorption in acid solution or in a non-polar solvent, and this fact is frequently taken as indicative of the existence of the open-chain structure. Thus, Buu-Hoï and his co-workers successfully demonstrated the presence in solution of the open forms of *o*-formylbenzoic acid (Buu-Hoï and Lin-che-Kin, *Compt. rend.*, 1939, **209**, 221) and opianic acid (Buu-Hoï, *ibid.*, 1941, **212**, 242), and these findings were confirmed by examination of suitable model compounds of known structure. The success of this technique is dependent on the compounds' being stable in dilute sodium hydroxide solution. Even if decomposition takes place slowly (*e.g.*, benzil-2-carboxylic acid, Fig. 6), and particularly if decomposition is rapid and extensive in the time normally taken in obtaining the spectral data (*e.g.*,

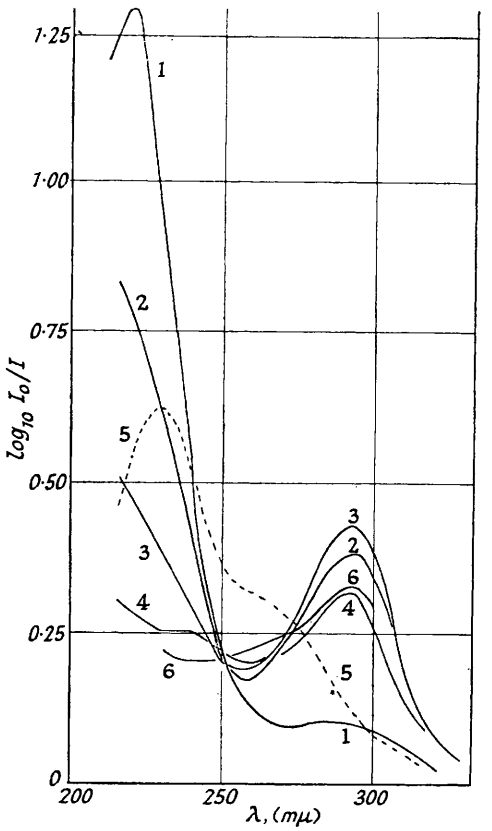
penicillic acid, Fig. 5), then erroneous conclusions may be drawn. Thus Shaw (*J. Amer. Chem. Soc.*, 1946, **68**, 2510), who obtained a curve similar to curve 4, Fig. 5, was led to conclude that penicillic acid exists in the keto-form in dilute sodium hydroxide whereas in fact it does not (Raphael, *J.*, 1947, 805; Ford *et al.*, *loc. cit.*) : the peak at 295 mμ is due to a decomposition product (Ford *et al.*, *loc. cit.*). Acidification of the alkaline solution after 4 hours (curve 5, Fig. 5) does not restore the absorption curve to its original form (curve 1) and penicillic acid is not recovered on ether-extraction of the acidified solution.

FIG. 4.



Phthalaldehyde (1) *in* n-*hexane*,
(2) *in* 0·1N-NaOH.

FIG. 5.



Penicillic acid;

1, *in* 0·1N-NaOH. l = 0·1 *cm.*
2, ,, *after* 1 *hour at* 26° ,,
3, ,, ,, 2 ,, ,,
4, ,, ,, 5 ,, ,,
5, 1 *ml. of soln.* (4) + 4 *ml. of* 0·1N-HCl, l = 1·0 *cm.*
6, *Soln.* (5) + 5 *ml. of* 0·1N-NaOH, ,,

The faintly yellow colour of a dilute alkaline solution of the colourless lactol form of benzil-2-carboxylic acid slowly deepens at room temperature (Fig. 6), but again this effect appears to be due to decomposition and not to the slow attainment of an equilibrium between the lactol and the keto ionic forms. The absorption spectrum of the yellow keto-form of benzil-2-carboxylic acid in dilute aqueous sodium hydroxide is identical with that of the lactol form in the same medium, the measurements being taken as quickly as possible after the solutions have been made up.

The gladiolic acid oxidation product, $C_{11}H_{10}O_6$ (IV), is stable to 0·1N-sodium hydroxide and behaves like the phthalaldehydic acids studied by Buu-Hoï and his co-workers (cf. bromo-opianic acid, Table 2). The absorption curve for its aqueous solution (Fig. 3) shows a

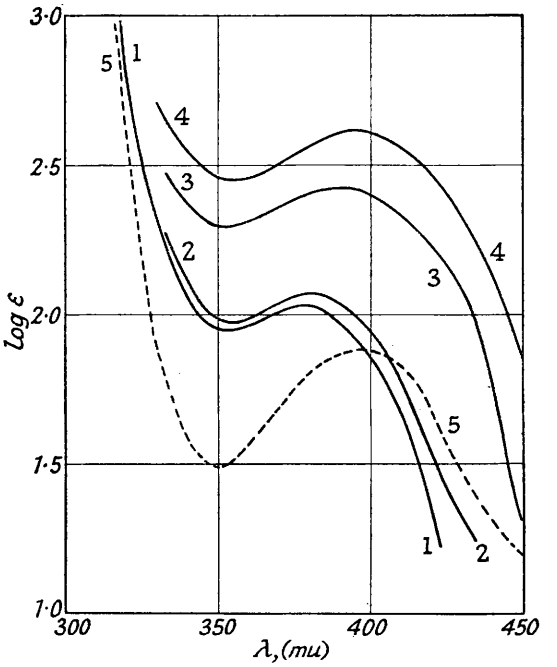


FIG. 6.

1, *Benzil-2-carboxylic acid (lactol or keto) in* 0·1N-NaOH.
2, *Benzil-2-carboxylic acid in* 0·1N-NaOH *after* 1 *day at* 20°.
3, *Benzil-2-carboxylic acid in* 0·1N-NaOH *after* 5 *days at* 20°.
4, *Benzil-2-carboxylic acid in* 0·1N-NaOH *after* 14 *days at* 20°.
5, *Benzil-2-carboxylic acid normal methyl ester.*

maximum at 296 mμ with an inflexion at 250 mμ; it is very similar to that of *iso*gladiolic acid (II; R = OH), and there is no doubt that the compound exists in the lactol form (IV*a*) in aqueous solution; but in 0·1N-sodium hydroxide the maxima are shifted to longer wave-lengths, demonstrating the presence of the open keto-form (IV*b*). Gladiolic acid,

TABLE 2. *Ultra-violet absorption data for gladiolic acid and its derivatives.*

Compound	Solvent	$\lambda_{max.}$	log ε
Deoxygladiolic acid (I*a*; R = H)	EtOH	306	3·62
		269	3·89
Acetylgladiolic acid (I*a*; R = OAc)	MeOH	306	3·48
		269	3·90
Methyl gladiolate (I*a*; R = OMe)	EtOH	305	3·50
		269	3·82
Ethyl gladiolate (I*a*; R = OEt)	EtOH	305	3·50
		269	3·91
*iso*Gladiolic acid (II; R = OH)	EtOH	298	3·72
		~245	3·93
Triacetylgladiolic acid hydrate (III; R = OAc)	EtOH	297	3·73
Oxidation product, $C_{11}H_{10}O_6$ (IV)	EtOH	300	3·40
		250	3·88
	H_2O	299	3·30
		~250	3·83
	0·1N-NaOH	310	3·40
		271	3·96
Bromo-opianic acid [a]	Aq. EtOH	312	3·5
		~250	3·5
	0·05N-NaOH	315	3·7
		280	4·1
Gladiolic acid	EtOH	305	3·48
		269	3·82
	H_2O	307	3·65
	(pH of soln., 3·9)	269	4·04
	0·1N-NaOH	276	3·08
Phthalaldehyde	*n*-Hexane	297	3·36
		255	3·95
	0·1N-NaOH	260 [b]	2·64 [b]
	McIlvaine buffer pH 2·97	261	2·88

[a] Buu-Hoï and Cagniant, *Compt. rend.*, 1941, **212**, 268. [b] Obtained within 30 mins. of the solution's being made up. Phthalaldehyde slowly undergoes decomposition in 0·1N-NaOH.

however, presents a rather different picture. The curves in water and in ethanol (Fig. 2) resemble those of deoxygladiolic acid, showing that gladiolic acid is in the lactol form (I*a*; R = OH) in these solvents; but in 0·1N-sodium hydroxide, in which gladiolic acid is stable, the spectrum being unaltered after 24 hours at 20°, only a weak absorption band at 276 mμ (log ε 3·1) is seen. In an earlier determination (Grove, *loc. cit.*), a second weak band was observed at 343 mμ (log ε 2·8) but recent work has shown that this band arose from a phenolic impurity which shows an intense absorption (log ε 4—4·5) in this region. Careful purification eliminates the band at 343 mμ, leaving only the weak band ot 276 mμ. Comparison of this curve with that given by phthalaldehyde in 0·1N-sodium hydroxide (Fig. 4) or McIlvaine buffer pH 2·97 suggests that gladiolic acid is in fact in a hydrated open-chain form (VI, or less probably VII) in alkaline solution. The shifting of the first absorption band to shorter wave-lengths with decrease in intensity in phthalaldehyde compared with the same compound in hexane (Fig. 4) is attributed to hydration involving both the formyl groups resulting in the suppression of the absorption pattern characteristic of a benzene nucleus conjugated with the C=O chromophore. Thus the absorption spectrum of phthalaldehyde in aqueous acid or alkaline solution (Table 2) is close to that of *o*-xylene ($\lambda_{max.}$ 262 mμ, log ε 2·47; Doub and Vandenbelt, *J. Amer. Chem. Soc.*, 1949, **71**, 2414), indicating the presence of a structure such as (VIII, R = R′ = H). Reliable data on a model compound strictly comparable with the gladiolic acid hydrate (VI) postulated above are not available, but Moser and Kohlenburg (*J.*, 1951, 804) have examined 2 : 6-dimethylbenzoic acid ($\lambda_{max.}$ 270 mμ, log ε 2·86) and 2 : 6-dimethoxybenzoic acid ($\lambda_{max.}$ 282 mμ, log ε 3·27) in ethanol. When account is taken of the small hypsochromic shift which occurs on replacement of the uncharged carboxyl substituent by the carboxyl ion, satisfactory correlation is obtained with the data for gladiolic acid. Thus the absorption spectrum of gladiolic acid in 0·1N-sodium hydroxide ($\lambda_{max.}$ 276 mμ, log ε 3·08) corresponds more closely with that of a 2 : 6-disubstituted benzoic acid than with that of a substituted phthalide [cf. triacetylgladiolic acid hydrate (III; R = OAc) ($\lambda_{max.}$ 297 mμ, log ε 3·73)]; and gladiolic acid is considered to be a hydrated keto-form (VI or VII), rather than a hydrated lactol form, in this medium. Of the two possible structures, (VI) is the more probable since the hydration phenomenon is only observed in the gladiolic acid anion where two formyl groups are present; there is no spectroscopic evidence for the hydration in aqueous solution of the single formyl substituent present in the lactol form (I*a*).

It may be concluded that whereas gladiolic acid is present in the lactol form in the solid state, an equilibrium between the lactol and the open-chain form exists in aqueous solution, pH being the determining factor. Similar conclusions apply to the oxidation product $C_{11}H_{10}O_6$. The esters of gladiolic acid described by Grove (*loc. cit.*) are shown conclusively to be *pseudo*-esters and the significance of this conclusion in the general problem of the correlation of chemical structure and biological activity in the gladiolic acid series of compounds will be discussed elsewhere.

I thank Dr. B. W. Bradford, Imperial Chemical Industries Limited, Billingham Division, and Mr. H. A. Willis, Imperial Chemical Industries Limited, Plastics Division, for ultra-violet and infra-red facilities, and Mrs. J. Zealley for assistance in the preparation of gladiolic acid hemihydrate.

Imperial Chemical Industries Limited, Butterwick Research Laboratories,
The Frythe, Welwyn, Herts. [*Received, April 29th*, 1952].