Chemistry of the Higher Fungi. Part V. The Structures of Nemotinic Acid and Nemotin.*

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The antibiotics nemotinic acid and nemotin have been separated from closely similar compounds produced by the same fungus and, despite their instability, have been characterised. Their high optical activity is due to the presence of asymmetrically substituted allenic groups. Nemotinic acid is readily converted into nemotin; the two compounds are shown to be 4-hydroxyundeca-5 : 6-diene-8 : 10-diynoic acid and the corresponding lactone.

IN 1950, workers at the New York Botanical Gardens (Anchel, Polatnick, and Kavanagh, *Arch. Biochem.*, 1950, **25**, 208; Kavanagh, Hervey, and Robbins, *Proc. Nat. Acad. Sci. U.S.A.*, 1950, **36**, 1) reported the isolation, from cultures of an unidentified Basidiomycete and also from *Poria corticola* and *Poria tenuis*, of two highly unsaturated antibiotics of

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* Part IV, Bu'Lock, *J.*, 1955, 575.

peculiar instability. To these the names nemotin and nemotinic acid were given. Both compounds showed characteristic ultraviolet absorption, with sharp maxima at a spacing of *ca.* 2000 cm.$^{-1}$, an interval previously observed with synthetic poly-ynes (Jones, Whiting, Armitage, Cook, and Entwistle, *Nature*, 1951, **168**, 900; Bohlmann, *Chem. Ber.*, 1951, **84**, 545, 785) and with polyacetylenic derivatives of methyl decanoate from various *Compositae* (Sörensen *et al.*, *Acta Chem. Scand.*, 1950, **4**, 416 and later papers). Both antibiotics were strongly dextrorotatory and their ultraviolet spectra showed drastic changes on alkali-treatment indicating rearrangements of the chromophoric groups. These observations of Anchel *et al.* suggested that nemotin and nemotinic acid might contain conjugated double and triple bonds and could conceivably owe their optical activity to asymmetrically substituted allenic groups. In view of the interest in these laboratories in synthetic poly-ynes, a study of these and related antibiotics was undertaken with a view to establishing their structures and structural relations. The subsequently published work of Celmer and Solomons (*J. Amer. Chem. Soc.*, 1952, **74**, 1870 and later papers) on mycomycin (trideca-3 : 5 : 7 : 8-tetraene-10 : 12-diynoic acid) and its isomerisation product (trideca-3 : 5-diene-7 : 9 : 11-triynoic acid) gave some support to the above views and provided a possible analogy for the alkali-instability of nemotinic acid.

Cultures of the antibiotic-producing fungus " B.841 " were kindly supplied by Dr. Anchel, and the fungus was grown in conditions essentially similar to those previously employed. Details of the culture-methods and observations of the effects of varying growth-conditions on polyacetylene production are reported elsewhere (Bu'Lock and Leadbeater, *Biochem. J.*, in the press). The mixture of compounds obtained by ethyl acetate extraction of the culture medium was resolved by Anchel *et al.* (*loc. cit.*), using counter-current distribution between benzene and water, into two fractions, neutral (" nemotin ") and acidic (" nemotinic acid "). When this separation was repeated, with a larger number of transfers studied quantitatively by spectrophotometry, the composite nature of both of these fractions was revealed. The acidic fraction was resolved directly into two components, and redistribution of the neutral material in an aqueous ethanol–*cyclo*hexane system showed that this also consisted of two substances. For the major acidic and neutral components the names nemotinic acid and nemotin are retained, and for the second components of each fraction the names odyssic acid and odyssin are proposed. Odyssic acid and odyssin bear the same relation to each other as do nemotinic acid and nemotin, and will be considered in a separate paper. The relative proportions of these four compounds in the extracts studied are given in Table 1.

TABLE 1. *Yields of polyacetylenes from the culture medium of the fungus* B.841.

Substance	Concn. in medium (mg./l.)	% of total
Nemotinic acid	110	67·5
Nemotin	14	8·5
Odyssic acid	34	21
Odyssin	5	3

All four compounds are unstable in the condensed phase and rapidly undergo polymerisation, particularly on exposure to light. This instability is most marked in nemotin and nemotinic acid; evaporation of solutions of these, even under reduced pressure (nitrogen) and in the dark, gives gums which cannot be completely redissolved. Because of the intractable nature of the compounds, special criteria of homogeneity were required for the present work. One such is provided by quantitative counter-current distribution in suitably chosen solvent systems. Both nemotinic and odyssic acids are obtained substantially pure from the initial benzene–water distribution, as shown by the shape of the distribution curve (Fig. 1), provided that the small intermediate fractions are rejected. The same criterion indicates homogeneity in the nemotin and odyssin obtained by redistribution of the neutral fraction (Fig. 2) and in the methyl ester obtained from nemotinic acid (Fig. 3). By weighing the residues remaining after evaporation of solutions of measured ultraviolet absorption, reproducible and consistent extinction coefficients were obtained for all compounds, and these are probably accurate to within $\pm 3\%$. Reproducible values for molecular rotations were obtained similarly, and the good values obtained for the equivalent weight of nemotinic acid by this means provided a check upon the method. Careful low-temperature crystallisation of nemotinic acid and its methyl ester at —60° in the dark gave solids, but since these reverted to oils below room temperature and polymerised on exposure to light, elementary analysis of the compounds in an undecomposed state was clearly impracticable. However, no change in weight was detected when a partly decomposed sample of nemotinic acid was completely polymerised by heating it in air at 100°, and elementary analyses of partly or wholly decomposed samples are accordingly given in the Experimental section. They are consistent with the presence of water as the sole major contaminant; complete drying of the polymeric resins is, as might be expected, difficult.

In a recent paper, Anchel and Cohen (*J. Biol. Chem.*, 1954, **208**, 319) recorded the hydrogenation of the neutral component (crude nemotin) and also of its alkali conversion product (nemotin-A) to *n*-undecanoic acid, identified as the *p*-toluidide. We have confirmed this observation, using pure nemotin free from odyssin. In addition to establishing the chain length of nemotin it suggested that nemotin contained a potential carboxyl group in a readily hydrogenolysed form. Anchel and Cohen do not record their yield of undecanoic acid, but we found that hydrogenolysis was incomplete and that the undecanoic acid was accompanied by considerable quantities of a lactone (see below).

Measurement of the infrared absorption spectrum of nemotin revealed the presence of acetylene, allene, acetylenic hydrogen, carbonyl, and methylene groups. The allene band at 1960 cm.$^{-1}$ is sharp and very characteristic (Sheppard and Simpson, *Quart. Rev.*, 1952, **6**, 1), whilst the presence of acetylenic hydrogen was confirmed by the formation of a white photosensitive precipitate with silver nitrate in ethanol. The carbonyl stretching band is at 1790 cm.$^{-1}$, a frequency intermediate between that expected for a saturated γ-lactone (*ca.* 1770 cm.$^{-1}$) and for a $\beta\gamma$-unsaturated γ-lactone (*ca.* 1800 cm.$^{-1}$) (Rasmussen and Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1077); the latter possibility is excluded by the absence of absorption near 1680 cm.$^{-1}$ where enol-lactones absorb strongly.

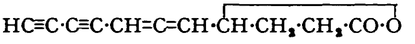
TABLE 2. *Ultraviolet absorption spectra.*

Substance	Absorption max. (Å) and log ε (in parentheses)				
Nemotinic acid	2785 (4·09)	2635 (4·19)	2495 (4·02)	2375 (3·75)	2090 (4·65)
Methyl nemotinate	2785 (4·09)	2635 (4·17)	2495 (3·99)	2375 (3·72)	2090 (4·64)
Nemotin	2780 (4·09)	2625 (4·19)	2490 (4·03)	2365 (3·79)	2085 (4·76)
Nemotin from nemotinic acid	2780 (4·09)	2625 (4·19)	2490 (4·03)	2365 (3·79)	2085 (4·74)

The ultraviolet absorption of nemotin (Table 2) is characteristic of the ene-diyne chromophore; the high-intensity short-wavelength absorption (log ε 4·76 at 2085 Å) distinguishes the spectrum from that of a conjugated diyne acid derivative, which would show similar (though less intense) absorption at the longer wavelengths. The low intensity (ε 3200) of the absorption at the minimum (*ca.* 2300 Å) renders unlikely the presence of any independent diene chromophore (cf. mycomycin, which shows independent absorption by two chromophores, each including one double bond of the allene group).

Combination of the infrared and ultraviolet absorption data with the hydrogenation evidence for a straight chain of eleven carbon atoms leads unambiguously to the structure (I) for nemotin, in which the ester grouping is allylic and hence subject to ready hydrogenolysis. The absence of large alkyl groups in (I) is confirmed by the low intensity (relative to the carbonyl or acetylenic hydrogen band) of the methylene CH stretching bands at 2840 and 2925 cm.$^{-1}$, and the absence of the band at 1380 cm.$^{-1}$ usually ascribed to $-CH_3$. The rather high frequency of the carbonyl band is probably due to the fact that the unsaturated chain of (I) contains carbon atoms in three different hybridisation states and thus exerts an effective dipole; the effect on the carbonyl frequency is then comparable with that observed in γ-acetoxy-γ-lactones and in cyanohydrin acetates (Rasmussen and Brattain, *loc. cit.*). Formula (I) contains two asymmetric centres, the carbon atom $C_{(4)}$ and the allene group, and nemotin is therefore one of the four possible stereoisomers. The structure proposed is completely in accordance with the alkali-catalysed conversion of nemotin into nemotin-A, to be discussed in a later paper.

The major polyacetylenic component of the extracts (Table 1), nemotinic acid, shows very similar ultraviolet absorption to nemotin, with the maxima uniformly displaced some 10 Å towards longer wavelengths (Table 2), and the infrared spectrum shows the presence of the same unsaturated groups. Thus the molecule also contains the unit $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{=}CH{\cdot}$ with the allene system not involved in further conjugation. The



$HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{=}CH{\cdot}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}O$

(I)

$HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{=}CH{\cdot}CH(OH){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$

(II)

acidity is due to a carboxyl group, whose C=O stretching frequency (1710 cm.$^{-1}$) and pK_a (4·80 in water) show that is is not conjugated. The methylene C–H stretching bands at 2860 and 2920 cm.$^{-1}$ have about the same intensity, relative to the ≡C–H band, as in nemotin; this, together with the equivalent weight (188), suggested a molecule of about the same size as nemotin, with $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{=}CH{\cdot}$ and $CH_2{\cdot}CO_2H$ as terminal groups.

The infrared spectrum of nemotinic acid shows several bands not present in that of nemotin, in particular, bands at 3500—3600 cm.$^{-1}$ superimposed on the very broad carboxyl O–H stretching bands. These suggest the presence of a hydroxyl group, and appear clearly in the infrared spectrum of methyl nemotinate (prepared from the acid and diazomethane). Acetylenic hydrogen was detected in the ester by the silver nitrate test. The attempted esterification of nemotinic acid by methanol–sulphuric acid gave a product shown by its infrared spectrum to be a mixture of methyl nemotinate and a γ-lactone. This mixture could be separated by counter-current distribution between aqueous ethanol and *cyclo*hexane; alternatively ester-free lactone could be obtained by the action of sulphuric acid in dry dioxan. The γ-lactone thus prepared is identical with natural nemotin in its ultraviolet and infrared absorption spectra, optical rotation, distribution characteristics, and reaction with alkali. The smooth conversion of nemotinic acid into nemotin requires the former to be one of the four possible stereoisomers of structure (II) which accords fully with the spectroscopic and other evidence discussed above, and also with its reaction with alkali, which will be discussed in a later paper.

Chemical evidence for the structure (II) would substantiate also the structure (I) for nemotin, and it was found possible to prove the chain length and the position of the hydroxyl group by hydrogenation. Direct hydrogenation of nemotinic acid gave a partly lactonised hydroxy-acid, whilst methyl nemotinate similarly gave the methyl ester of a hydroxy-acid. Distillation of either product under reduced pressure gave the same γ-lactone (infrared absorption band at 1775 cm.$^{-1}$), an oil of very characteristic odour. This odour is the same as that of (±)-undecano-γ-lactone, readily prepared by treatment of undecylenic acid with concentrated sulphuric acid at 100°, and the two compounds show identical infrared absorption spectra in carbon disulphide solution. However, the lactone prepared from the antibiotics is optically active ($[\alpha]_D$ +31°) and proof of its formulation as γ-undecanolactone required conversion into an optically inactive material. The hydroxy-amide prepared from the (+)-lactone is crystallographically distinct from the (±)-derivative, but oxidation of the ammonolysis products gave identical samples of 4-oxoundecanamide.

EXPERIMENTAL

So far as possible, unsaturated compounds were handled under nitrogen and in darkness or diffused light. Solutions were concentrated under reduced pressure at 40° or less, and for storage were kept as dilute as practicable; at concentrations greater than about 0·3% decomposition was appreciable in 24 hr. Ultraviolet absorption spectra (Unicam SP-500 Spectrophotometer) and optical rotations were measured in ethanol; for the measurements of infrared spectra, solutions were evaporated in darkness at −50° and the soluble part of the residues taken up in carbon tetrachloride or carbon disulphide. The concentration of polyacetylenes in crude solutions was calculated from the observed difference between optical densities at the longest-wavelength maximum and minimum (*ca.* 2780 and 2710 Å respectively). M. p.s were determined on the Kofler block, and mixed m. p.s confirmed by fusion analysis.

Isolation of Polyacetylenes.—The growth conditions employed are described elsewhere (Bu'Lock and Leadbeater, *loc. cit.*). In a typical experiment, 7 l. of culture medium (containing *ca.* 1200 mg. of mixed polyacetylenes) was extracted once with one-fifth and twice with one-tenth its volume of ethyl acetate, and the combined extracts, after spectroscopic assay, were evaporated to *ca.* 400 c.c.; water (400 c.c.) was then added and the evaporation continued until about 100 c.c. of ethyl acetate remained. Ethanol (100 c.c.) was then added to facilitate transfer of polyacetylenes to the aqueous phase, and all organic solvents were finally removed by continued evaporation. The aqueous solution thus obtained contained practically all the original polyacetylenes of the extracts; after storage overnight at 0° it was filtered and placed in the first ten tubes of a 60-plate Craig-type counter-current distribution apparatus (volumes of upper and lower phases 40 c.c.) and fractionated with benzene. Generally about 100 transfers were employed; the neutral polyacetylenes appeared in the first benzene fractions (see below) and the distribution was stopped when odyssic acid began to appear in the benzene fractions being taken off. The odyssic acid was then found near the end of the distribution and the nemotinic acid near the middle (Fig. 1). After discarding intermediate fractions, the total contents of appropriate tubes were evaporated until all the benzene was removed, and the aqueous solutions acidified (pH 4) and extracted rapidly with two portions (one-fifth volume) of peroxide-free ether. The acids were stored in ether at −5° until required (in ethanol some esterification took place). The first benzene fractions to be removed from the distribution were combined and the neutral polyacetylenes redistributed between *cyclo*hexane and water containing 30% of ethanol. After 30 transfers, a good separation of nemotin and odyssin was achieved (the nemotin being more soluble in the aqueous layer) though some material was lost in the intermediate (mixed) fractions (Fig. 2). From 1200 mg. of total polyacetylenes there were obtained the following yields of purified polyacetylenes: nemotinic acid 660 mg., odyssic acid 180 mg., nemotin 105 mg., odyssin 15 mg., total 960 mg.

FIG. 1.

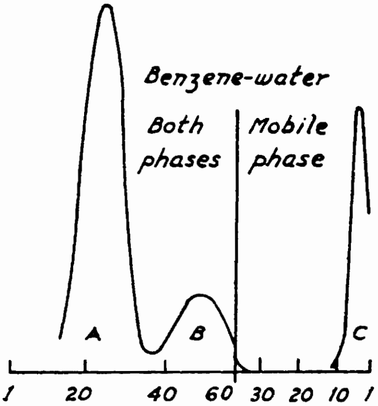


FIG. 2.

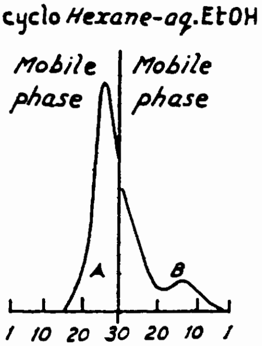
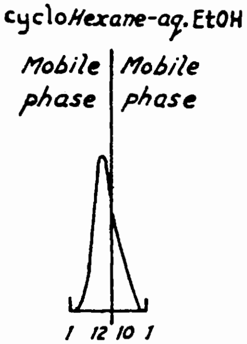


FIG. 3.



Counter-current distribution experiments. (1) *Separation of nemotinic acid* (*A*) *and odyssic acid* (*B*) *from the neutral fraction* (*C*). (2) *Resolution of the neutral fraction into nemotin* (*A*) *and odyssin* (*B*). (3) *Purification of methyl nemotinate. For each Figure the curve to the left of the vertical line refers to concentrations* (*arbitrary units*) *in successive tubes of the apparatus; that to the right refers to concentrations in fractions taken successively from the last tube of the apparatus.*

Physical Constants.—Aliquot samples (10 c.c. containing *ca.* 8 mg.) of standard solutions of the purified compounds were evaporated to dryness in tared flasks, and the solid residues were weighed. The average of three such weighings was taken, variation being less than ±5%. The samples were usually partly decomposed; there was no detectable change in weight when decomposition was completed by heating a sample at 100°. For nemotinic acid, analyses were performed on the resin (Found: C, 68·3; H, 5·7. Calc. for $C_{11}H_{10}O_3$: C, 69·5; H, 5·3. Calc. for $C_{11}H_{10}O_3,0{\cdot}2H_2O$: C, 68·2; H, 5·4%), also for nemotin (Found: C, 72·6; H, 5·2. Calc. for $C_{11}H_8O_2$: C, 76·7; H, 4·7. Calc. for $C_{11}H_8O_2,0{\cdot}5H_2O$: C, 72·9; H, 5·0%). The following physical constants were then determined using the standard solutions: for nemotinic acid, $[\alpha]_D^{17}$ +320° (*c*, 0·2); equiv., 186, 188 (Calc. for $C_{11}H_{10}O_3$: 190); pK_a (in water) 4·80; ultraviolet absorption, Table 2; for nemotin, $[\alpha]_D^{17}$ +380° (*c*, 0·3); partition coefficient between *cyclo*hexane and water containing 30% of ethanol by volume, 0·88; ultraviolet absorption, Table 2.

Esterification of Nemotinic Acid.—(*a*) *With methanol and sulphuric acid.* A solution of nemotinic acid (200 mg.) and sulphuric acid (0·9 c.c.) in methanol (30 c.c.) was kept in the dark at 20° for 3 days. The solution was then poured into water and extracted with ether (4 × 50 c.c.), and the combined extracts were washed with sodium hydrogen carbonate solution and water and then dried. The product showed strong infrared absorption at 1790 (lactone) and 1735 cm.$^{-1}$ (ester), and the mixture was resolved by counter-current distribution between *cyclo*hexane and aqueous ethanol.

(b) *With diazomethane.* Nemotinic acid (190 mg.) in ether solution was treated with a slight excess of distilled diazomethane in ether. After 15 min., excess of reagent was decomposed with acetic acid, and the solution was washed with sodium hydrogen carbonate and water and then dried. The infrared absorption of the product showed that no lactonisation had occurred, and the homogeneity of the product was demonstrated by counter-current distribution (Fig. 3). Physical constants for methyl nemotinate were determined as for nemotin : $[\alpha]_D^{17}$ +300° (*c*, 0·35); partition coefficient between *cyclo*hexane and water containing 10% by volume of ethanol, 1·02; ultraviolet absorption, Table 2. Material obtained by evaporation of solutions of purified ester was analysed (Found : C, 68·8; H, 6·0. Calc. for $C_{12}H_{12}O_3$: C, 70·6; H, 5·9. Calc. for $C_{12}H_{12}O_3,0{\cdot}3H_2O$: C, 68·8; H, 6·0%).

Conversion of Nemotinic Acid into Nemotin.—Nemotinic acid (600 mg.) was transferred from ether into pure dry dioxan (480 c.c.) by evaporating off the ether, and concentrated sulphuric acid (15 c.c.) was added. After 4 days in the dark at room temperature, water (200 c.c.) was added and the solution brought to pH 4 with sodium hydrogen carbonate solution. The precipitated sodium sulphate was filtered off and washed, and the combined filtrate and washings were evaporated to 200 c.c. Water (2 l.) was added and the solution was extracted twice with 400 c.c. portions of ether. The ether extracts contained 470 mg. of neutral polyacetylenes. This material, purified as described for nemotin, gave 350 mg. of pure lactone with $[\alpha]_D^{17}$ +350° (*c*, 0·3) and infrared and ultraviolet absorption spectra identical with those of nemotin. The hydrogenation and alkali-isomerisation behaviour of nemotin were all confirmed with lactonised nemotinic acid.

Hydrogenation of Nemotinic Acid.—Hydrogenation of a solution of the acid (55 mg.) in acetic acid (25 c.c.) over platinum (from 50 mg. of oxide) proceeded with the uptake of *ca.* 5·0 mols. of hydrogen. The solution was filtered and evaporated to give an oil which after distillation under reduced pressure had infrared absorption identical with that of synthetic (±)-γ-undecanolactone and showed $[\alpha]_D^{17}$ +29° (*c*, 1·7), n_D^{20} 1·4540. The lactone (45 mg.) was dissolved in ethanol and the solution was kept saturated with ammonia for 4 days. Evaporation of the solution gave a crude hydroxy-amide which was oxidised directly in acetone solution with excess of chromic acid. Water was added to the reaction mixture and this was extracted continuously with ether. Evaporation of the ether and recrystallisation from benzene–light petroleum gave 4-oxoundecanamide (18 mg.), m. p. 130—131°, undepressed on admixture with synthetic material (see below).

Hydrogenation of Methyl Nemotinate.—Hydrogenation of the ester (105 mg.) in ethanol over platinum proceeded with the uptake of 5·4 mols. of hydrogen to give, after filtration and evaporation, an oil (106 mg.) with the infrared absorption of a hydroxy-ester. On distillation this gave the above (+)-γ-undecanolactone. Ammonolysis of the ester, as described for the lactone, and oxidation of the crude hydroxy-amide (95 mg.) gave 4-oxoundecanamide (35 mg. after recrystallisation) as above.

Hydrogenation of Nemotin with Partial Hydrogenolysis.—Hydrogenation of nemotin (100 mg.) in acetic acid (50 c.c.) containing a trace of perchloric acid over a platinum catalyst proceeded with the uptake of 6·4 mols. of hydrogen; evaporation of the solution gave an oil with infrared absorption bands at 1705 (strong) and 1780 cm.$^{-1}$ (medium), ascribed to acid and lactone respectively. Excess of phosphorus pentachloride was added to the mixture and the products were taken up in dry ether. To the solution was added *p*-toluidine (0·2 g.) in ether, after which the solution was washed successively with dilute hydrochloric acid, sodium carbonate solution, and water. Evaporation of the dried ether solution gave a solid (86 mg.), which when crystallised from light petroleum gave the *p*-toluidide of undecanoic acid, m. p. 78°, undepressed on admixture with authentic material.

(±)-*4-Hydroxyundecanamide and 4-Oxoundecanamide.*—γ-Undecanolactone, b. p. 164—166°/13 mm., n_D^{20} 1·4514, prepared by the method of Grün and Wirth (*Ber.*, 1922, **55**, 2217), was treated with an excess of a saturated solution of ammonia in methanol to give (±)-4-*hydroxyundecanamide*, which, crystallised from ethyl acetate–light petroleum, was found to be dimorphous with m. p. 80° and 98—99° (Found : C, 65·65; H, 11·3; N, 7·3. $C_{11}H_{23}O_2N$ requires C, 65·6; H, 11·5; N, 7·0%). Oxidation of this hydroxy-amide in acetone with excess of chromic acid and continuous extraction of the product from the diluted reaction mixture with ether gave, after crystallisation from ethyl acetate–light petroleum, 4-*oxoundecanamide*, m. p. 130—131° (Found: C, 66·45; H, 10·25; N, 7·05. $C_{11}H_{21}O_2N$ requires C, 66·3; H, 10·6; N, 7·05%).

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