while a large excess of H_2O was added. The solution was extracted with EtOAc to separate the liberated organic acid which was further purified by taking it up in 2M $KHCO_3$, acidifying, and again extracting with EtOAc. This solution was washed with H_2O until neutral, dried (Na_2SO_4), and filtered. After evaporation of the solvent the residue was crystallized from EtOH/H_2O, giving analytically pure Bz-L-Phe, 227 mg (84%). M.p. 134–134.5° (Lit. [9] m.p. 142–143°). $[\alpha]_D^{25} = +37.96°$ and $[\alpha]_{546}^{25} = +45.92°$ ($c = 1.6$, dioxane) (Lit. [9] $[\alpha]_D^{25} = +38.74°$ and $[\alpha]_{546}^{25} = +45.73°$).

$C_{16}H_{15}NO_3$ (269.2) Calc. C 71.36 H 5.61 N 5.20% Found C 71.52 H 5.75 N 5.20%

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62. A Chemical Study of *Burley* Tobacco Flavour (*Nicotiana tabacum* L.) V. Identification and Synthesis of the Novel Terpenoid Alkaloids 1,3,6,6-Tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one and 3,6,6-Trimethyl-5,6-dihydro-7*H*-2-pyrindin-7-one[1][2]

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(20. XII. 74)

Summary. GLC. allowed the isolation of 1,3,6,6-tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one (*A*) and 3,6,6-trimethyl-5,6-dihydro-7*H*-2-pyrindin-7-one (*D*) from *Burley* tobacco condensate (about 0.1% each). The structures and syntheses of these novel terpenoid alkaloids are described, and a possible way for their formation in tobacco is suggested.

The new compounds **A** and **D** were isolated[3] by subjecting subfractions B2-PN-j and B3-PN-i from *Burley* tobacco condensate to GLC. separations[4]. The former subfraction was found to contain 3.21% of **D**, the latter 0.37% of **D** and 1.52% of **A**,

[1] For the 4th publication of this series see [1].

[2] Part of this work was included in a paper presented by *E. D.* at the VIth International Congress of Essential Oils (San Francisco, Calif., Sept. 8–12, 1974).

[3] We thank Mr. *D. Berthet* for this isolation work.

[4] *Burley* tobacco condensate and subfraction B2-PN-j were obtained and investigated as previously described [2]. Compound **D** was eluted between 1,5,5-trimethyl-9-oxabicyclo-[4.3.0]nonan-3-one and nicotyrine on Carbowax at 220° (see *Scheme 13* in paper [2]). The preparation and study of subfraction B3-PN-i will be described in a future paper.

this corresponding to a total amount of about 0.1% of each alkaloid in the whole tobacco condensate used for our investigation.

1. *1,3,6,6-Tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one* (**A**). a) *Structure elucidation.* High resolution mass spectrometry indicated this new tobacco constituent to have a molecular weight of 203.127[5]) in agreement with the formula $C_{13}H_{17}NO$. Beside the parent ion, the mass spectrum of **A** exhibits a *M*-56 fragment at *m/e* 147 corresponding to the loss of isobutene (Fig. 1). IR. spectrometry disclosed the presence of an α,β-unsaturated ketone absorbing at 1670 cm^{-1} (Fig. 1), associated at least in part with three UV. maxima at 212, 248 and 280 nm in ethanol ($\varepsilon = 17700, 9800, 4850$). Although the NMR. spectrum (Fig. 1) displays only five singlets, it was soon noted that the lower field signal at $\delta = 6.90$ appears at the expected value for a pyridine β proton [3]. The four other singlets were tentatively assigned to two geminal methyls ($\delta = 1.08$, 6H), two other methyls presumably located α to the nitrogen atom in a pyridine ring ($\delta = 2.54, 2.85, 2\times3H$), and two methylene groups located α to either the pyridine ring or the ketone function ($\delta = 2.54, 2.80, 2\times2H$). Further details were revealed by the NMR. spectrum of the corresponding alcohol **B** obtained by hydrogenating the alkaloid over a platinum catalyst. In this spectrum, there is an obvious *ABX* coupling between the $>CH_X$-OH group and one adjacent methylene group. Thus, the proton *X* appears as a triplet ($J_{XA} \simeq J_{XB} \simeq 5.5$ Hz) at $\delta = 4.95$, whereas the methylene protons *A* and *B* give rise to a doublet of quartets centered at $\delta = 1.90$ ($J_{AX} \simeq J_{BX} \simeq 5.5$ Hz, $J_{AB} = 14$ Hz). The second methylene group in **B** appears at $\delta = 2.52$ as a simple *AB* quartet ($J \simeq 18$ Hz) partly hidden behind a methyl singlet. All these results, confirmed by decoupling and lanthanide induced shift experiments[6]), left only **A** or **C** as possible structures for the novel tobacco alkaloid. Since the NMR.-spectrum (Fig. 1) of this substance exhibits a singlet at $\delta = 2.85$ attributable to a methyl group deshielded by the carbonyl function, the former structure, **A**, was thought to be correct.

b) *Synthesis.* Compound **A** was first synthesized from γ-cyclogeraniolene (**1**) employing the two-fold acetylation procedure of *Balaban* & *Nenitzescu* [4] (Ac_2O/$HClO_4$). Ammonolysis of the intermediate pyrylium salt **2** led to the tetrahydro-isoquinoline **3** (accompanied by some of isomer **4**) which was finally oxidized by *t*-butyl chromate [5]. However, this «benzylic» oxidation lacked regio-selectivity and afforded a 1.0:1.3:1.0 mixture of **A** together with its isomer **5** and the diketone **6**. Considering that isophorone (**7**) is known to react at both its α and γ-methyl positions with electrophiles [6] [7], we felt this ketone might undergo the same exhaustive acetylation as the olefin **1**. This would provide a shorter, non-oxidative synthesis of **A** which should result directly from the ammonolysis of the hypothetical intermediate **8**. A modest yield of **A** (5.4%, isolated, pure, m.p. 34–35°) was indeed obtained by simply treating isophorone (**7**) with acetic anhydride/perchloric acid and ammonium hydroxide, successively. No attempt to improve this yield was made as this simple procedure met our practical requirements.

[5]) We wish to express our thanks to Prof. *A. Buchs* (University of Geneva), in the laboratory of whom this determination was made.

[6]) We are much indebted to Dr. *B. Willhalm* and to Mr. *W. Thommen* (*Firmenich* SA, Geneva) for having conducted these experiments.

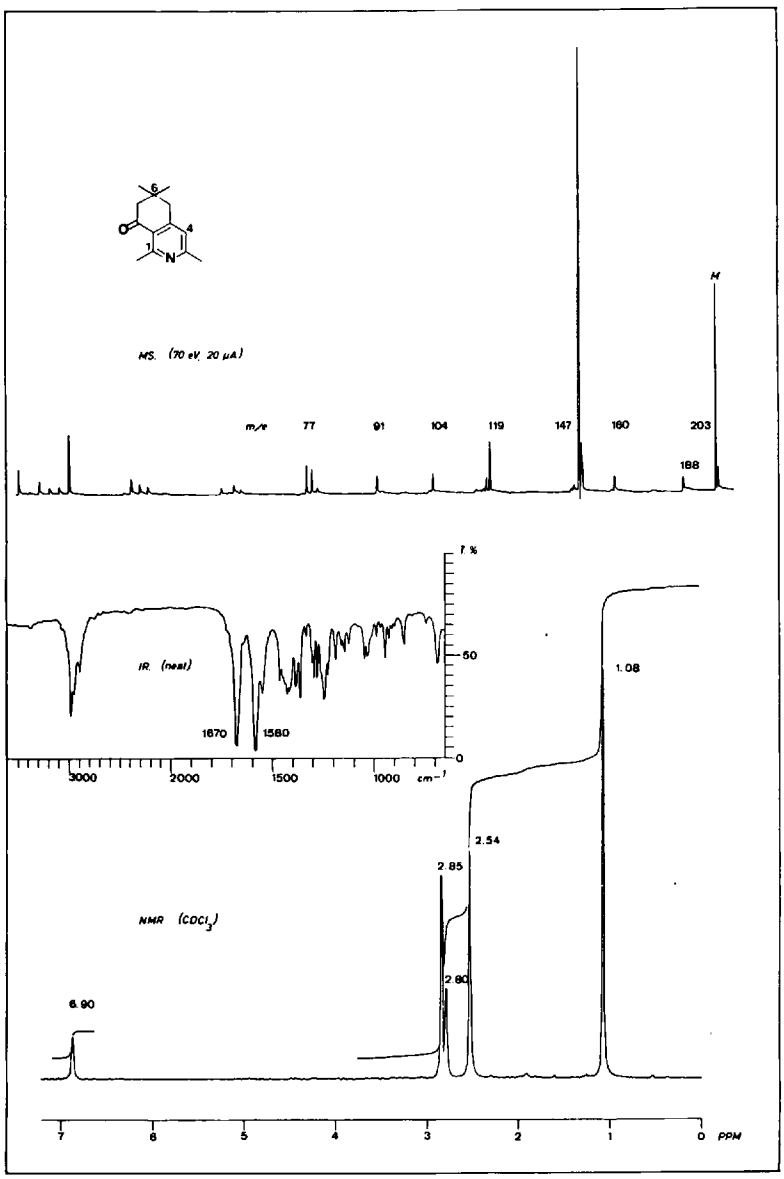
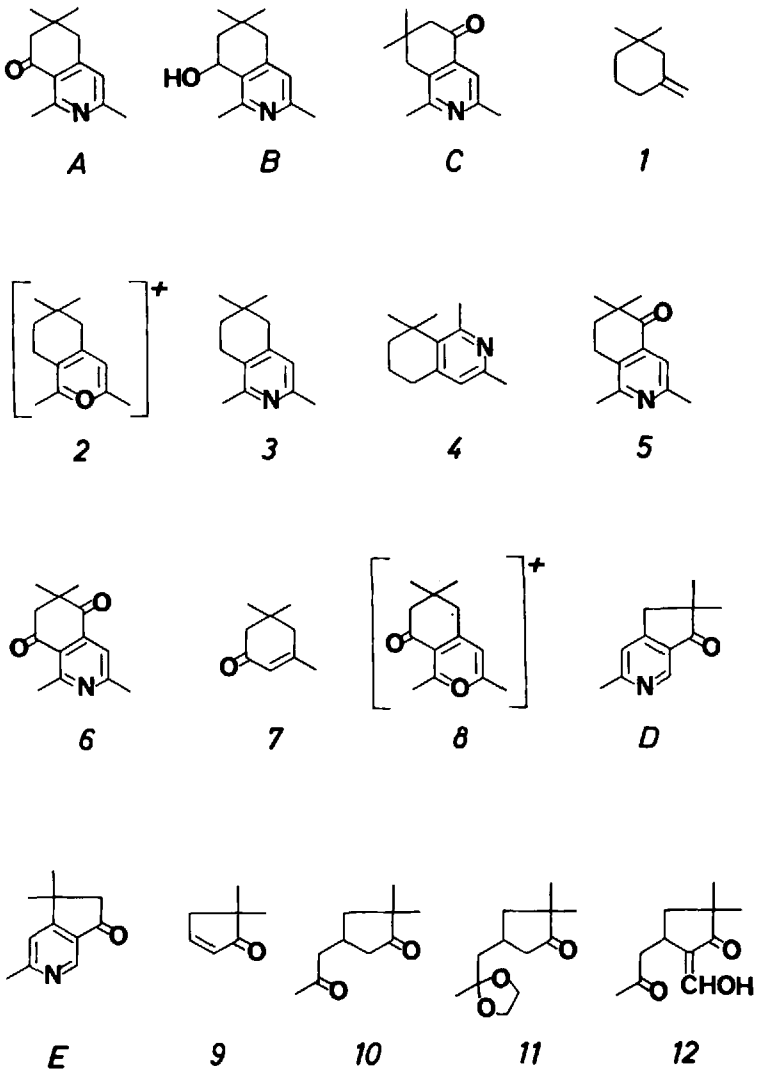


Fig. 1. *Mass, IR. and NMR. spectra of 1,3,6,6-tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one* (**A**)

Synthetic 1,3,6,6-tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one (**A**), prepared by either above routes was found to be identical in every respect (IR., UV., MS., NMR.) with the natural tobacco isolate.

*2. 3,6,6-Tetramethyl-5,6-dihydro-7*H*-2-pyrindin-7-one* (**D**). a) *Structure elucidation.* High resolution mass spectrometry showed this second tobacco alkaloid to have a molecular weight of 175.099[5]) corresponding to the formula $C_{11}H_{13}NO$. The mass



spectrum (Fig. 2) is characterized by a $M-15$ base peak at m/e 160. The IR.-spectrum (Fig. 2) displays a carbonyl band at 1705 cm^{-1} which, considering the strong UV.-absorption of the alkaloid at 245 nm (ε = 10700, ethanol), was best attributed to a conjugated cyclopentenone. The five singlets which appear in the NMR.-spectrum (Fig. 2) were respectively attributed to two geminal methyls (δ = 1,26, 6H), one methyl located α to the nitrogen atom in a pyridine ring (δ = 2.67, 3H), one methylene group α to either the pyridine ring or the ketone function (δ = 2.99, 2H), one pyridine β proton (δ = 7.27, 1H); of particular interest is the last, low-field singlet at δ = 8.89 (1H), which strongly suggested a pyridine α proton further deshielded by the cyclopentenone carbonyl group (normal δ value for pyridine α protons $\simeq$ 8.5). This detail

together with the preceding results led us to propose structures **D** or **E** for the novel alkaloid. While both these structures are in accord with the virtual absence of coupling between the two pyridine protons (expected $J_{1,4}$ value = 0.7 to 0.9 Hz [8]), **D** was thought to be correct since it agrees especially well with the chemical shifts of the geminal methyls (δ = 1.26) and of the methylene group (δ = 2.99) of the alkaloid.

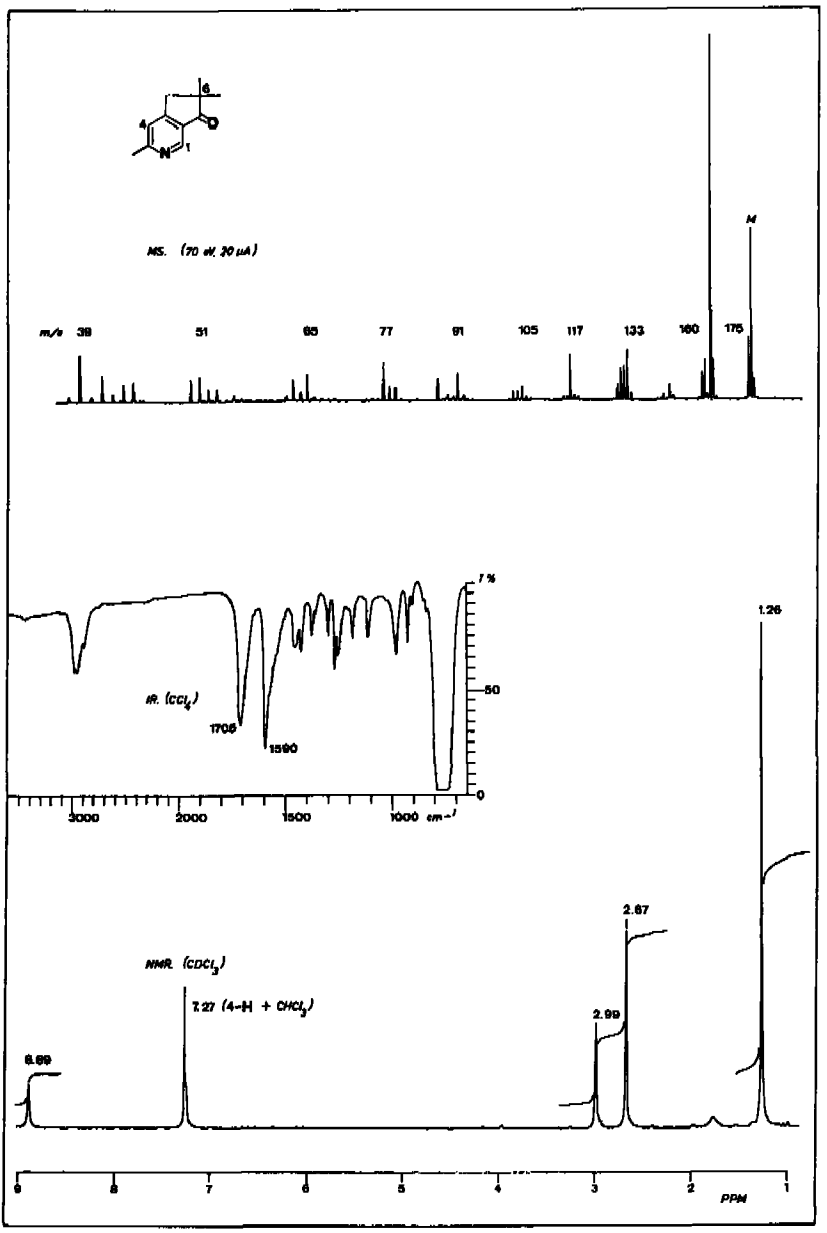
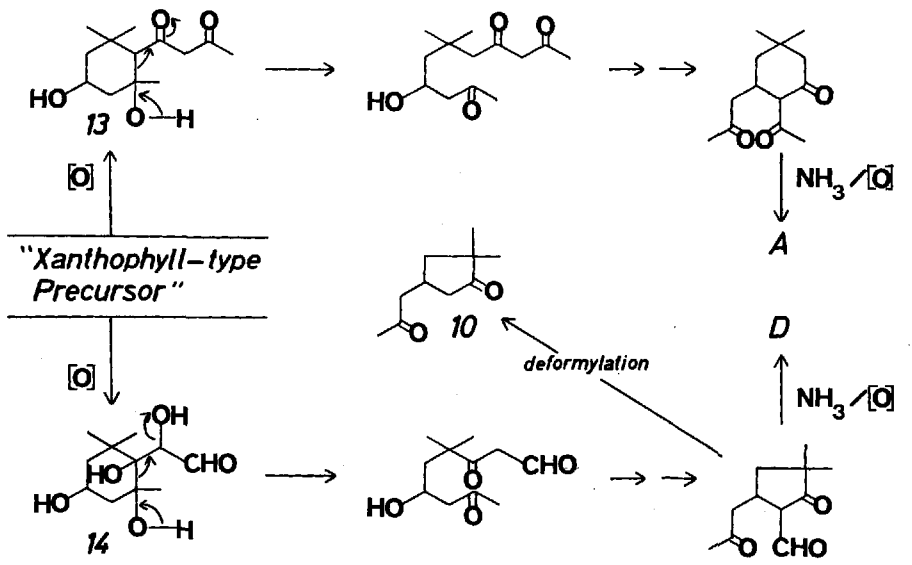


Fig. 2. *Mass, IR. and NMR. spectra of 3,6,6-trimethyl-5,6-dihydro-7*H*-2-pyridin-7-one* (**D**)

b) *Synthesis.* Tobacco alkaloid **D** was synthesized *via Michael* addition of ethyl acetoacetate to 5,5-dimethylcyclopent-2-en-1-one (**9**) [9], selective acetalization [10] of the methyl-ketone function in **10**, formylation [11] of acetal **11**, hydrolysis of the formed product, cyclization of the crude hydroxymethylene derivative **12** with ammonium acetate and, finally, nitrous acid oxidation. This afforded an overall yield of about 5% of pure 3,6,6-trimethyl-5,6-dihydro-7*H*-2-pyrindin-7-one (**D**, m.p. 76–77°) which exhibited IR., UV., NMR., and mass spectra identical to those of the natural tobacco isolate. The diketo intermediate **10** was also found to occur in *Burley* tobacco condensate[7]), a fact that might have some significance with respect to the chemistry of **D** in tobacco (*v. infra*).

3. *Formation of compounds* **A** *and* **D** *in tobacco.* Their unusual structures suggest that these compounds do not arise from a straightforward biosynthetic process, but that they are more likely formed *via* a number of degradative enzymatic and chemical reactions during the aerobic treatment of the tobacco leaves (curing, aging). Alkaloids **A** and **D** probably originate from the exhaustive oxidation of higher terpene precursors such as *xanthophylls*. They might for instance result from successive ring cleavage, intramolecular *Michael*, and ammonia cyclization reactions of hypothetical, highly oxygenated xanthophyll metabolites **13** and **14** (*Scheme*), a route that would also account for the formation of diketone **10**. This view is supported by the recent work of *Ohloff & Skorianetz* [12–14] in the ionone series, which provides evidence that polyols analogous to **14** can indeed undergo ring cleavage with acids (*English-Zimmermann* fragmentation). Many other C_9, C_{11} and C_{13} *Burley* tobacco constituents having regular, monocyclic terpene structures, were formerly thought to be derived

Scheme. *A possible way to the formation of* **A**, **D** *and* **10** *from carotenoid precursors*



[7]) *E. Demole & C. Demole*, unpublished results.

from the breakdown of carotenoids [2] [15]. This assumption has also received additional support since *Ohloff et al.* [16] and *Sakan et al.* [17] have recently put forward proposals regarding the possible formation of the natural damascones and damascenone from *neoxanthin-type precursors.*

Experimental Part

The spectra were measured on the instruments already mentioned [18] (the NMR. spectra were measured in $CDCl_3$ unless otherwise stated). The GLC. separations were performed on a gas chromatograph *Aerograph*, Model 1820-3 (*Varian Aerograph* AG). The melting points were determined on a hot-stage microscope and are not corrected.

1. *1,3,6,6-Tetramethyl-5,6,7,8-tetrahydroisoquinolin-8-one* (*A*). a) *From γ-cyclogeraniolene* (**1**). *n*-Butyl-lithium (3.85 g, 60 mmol, 14% solution in hexane) was added at 20° under N_2 to a slurry of triphenylmethylphosphonium bromide (23.6 g, 66 mmol) in 100 ml of anhydrous ether. After 2 h of further stirring, 3,3-dimethylcyclohexanone [19] (6.3 g, 50 mmol, in 20 ml of anhydrous tetrahydrofuran) was added to the mixture under external cooling with ice-water. The reaction mixture was further stirred for 2 h, kept overnight at 20°, heated for 1 h at 60° (ether solvent was replaced by tetrahydrofuran), and subjected to usual work up. There was thus obtained 4.1 g (66%) of γ-cyclogeraniolene (**1**), b.p. 128°/730 Torr. – NMR. (CCl_4): δ = 0.85 (6 H, *s*); 1.05–1.80 (4 H, *m*); 1.80–2.30 (4 H, *m*); 4.58 (2 H, *d*, J = ~4.5 Hz).

70% Perchloric acid (5.4 ml, 63 mmol) was cautiously added to γ-cyclogeraniolene (**1**) (3.9 g, 31.4 mmol) in acetic anhydride (32 g) at 100° [4]. A vigorous reaction occurred, and after 3 h of further heating at 100°, the mixture was poured into water, mixed with an excess of aqueous ammonia (with respect to acetic anhydride), and finally extracted twice with ether. The combined organic layers were filtered and extracted 3 times with 10% hydrochloric acid to separate the basic compounds. These were isolated in the usual manner and distilled (0.001 Torr): Fr. 1, b.p. 80–85°, 0,16 g; Fr. 2, b.p. 85–88°, 1.30 g; Fr. 3, b.p. 90–110°, 0,37 g. The combined fractions represented 30.8% yield (GLC.) of a ~19:1 mixture of tetrahydro-isoquinolines **3** and **4** (15% silicone oil, 200°, 2.5 m column; approx. R_T = 1.00 and 1.08). A sample of pure tetrahydro-isoquinoline **3** collected by GLC. had d_4^{20} = 0.9675; n_D^{20} = 1.5212. – IR. (neat): ν = 1560, 1590 cm^{-1}. – MS.: M^+ = 189, base peak *m/e* 133. – NMR.: δ = 0.94 (6 H, *s*); 1.60 (2 H, *t*, J = 6.5 Hz); 2.60 (2 H, *t*, J = 6.5 Hz); 2.40 (8 H, *s*), 6.65 (1 H, *s*).

$C_{13}H_{19}N$ (189.30) Calc. C 82.48 H 10.12 N 7.40% Found C 82.53 H 10.17 N 7.30%

Minor isomer **4**: IR. (CCl_4): ν = 1540, 1580 cm^{-1}. – MS.: M^+ = 189, base peak *m/e* 174. – NMR.: δ = 1.38 (6 H, *s*); 1.6–2.0 (4 H, *m*); 2.40 (3 H, *s*); 2.68 (5 H, *s* + *t*); 6.70 (1 H, *s*). A mixture of *t*-butyl chromate reagent (1.8 ml) [5], acetic acid (0.6 ml) and acetic anhydride (0.25 ml) was added to a refluxing solution of **3** (120 mg, 0.635 mmol) in CCl_4 (1.2 ml). After 2 h further refluxing, the mixture was cooled to 0°, and a solution of 375 mg of oxalic acid in 3.8 ml of water was added, followed by 260 mg of the pure acid. After further stirring for 2 h, the mixture was made alkaline and extracted twice with ether. GLC. (15% Carbowax, 220°, 2.5 m column) indicated that the product was a mixture of ~65% of starting tetrahydro-isoquinoline **3**, 10% of the desired *tetrahydro-isoquinolinone* ***A***, 14% of isomer **5** and 11% of diketo derivative **6**. Compound **5**: IR. (CCl_4): ν = 1560, 1580, 1680 cm^{-1}. – MS.: M^+ = 203, base peak *m/e* 119. – NMR.: δ = 1.16 (6 H, *s*); 1.97 (2 H, *t*, J = 6.5 Hz); 2.50 (6 H, *s*); 2.82 (2 H, *t*, J = 6.5 Hz); 7.48 (1 H, *s*).

Diketo derivative **6**: IR. (CCl_4): ν = 1570, 1680 cm^{-1}. – MS.: M^+ = 217, base peak *m/e* 202. – NMR.: δ = 1.27 (6 H, *s*); 2.63 (3 H, *s*); 2.86 (5 H, broad *s*); 7.55 (1 H, *s*).

b) *From isophorone* (**7**). A stirred solution of isophorone (**7**) (27.6 g, 0.20 mol) in acetic anhydride (200 ml) was heated to 100°, when perchloric acid (70%, 34 ml, 0.40 mol) was added over 2 h. After 2 h of further heating at 100°, the mixture was poured into ice-water, and 25% aqueous ammonia solution (800 ml) added with cooling so as to maintain the reaction temperature below 30°. The mixture was stirred overnight at room temperature, extracted twice with ether, and the combined organic layers filtered and washed 3 times with 10% hydrochloric acid. The basic fraction (11 g) isolated in the usual way was first filtered through a column of 110 g of silica gel[8])

[8]) «Kieselgel 0.05–0.2 mm für die Säulen-Chromatographie» (*Merck* AG).

using ethyl acetate as solvent. The eluted material (6 g) was then chromatographed on 120 g of silica gel as follows:

Fractions	ml EtOAc	mg eluted
1	125	2015
2	75	3460
3	135	940

Fraction 2 was distilled (0.001 Torr): Fr. 2a, b.p. 95°, 1.41 g; Fr. 2b, b.p. 95–105°, 0.80 g (yield Fr. 2a + 2b = 5.4% of **A**). GLC. (5% Carbowax, 220°, 2.5 m column) indicated that Fr. 2a was pure *1,3,6,6-tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one* (**A**), the properties of which were discussed above in the theoretical part.

$C_{12}H_{17}NO$ (203.28) Calc. C 76.81 H 8.43 N 6.89% Found C 76.64 H 8.43 N 7.02%

2. *3,6,6-Trimethyl-5,6-dihydro-7*H*-2-pyrindin-7-one* (**D**). A mixture of 5,5-dimethyl-cyclopent-2-en-1-one (**9**) [9] (1.28 g, 11.6 mmol), ethyl acetoacetate (3 ml), and sodium methoxide (210 mg, 3.9 mmol) in anhydrous ethanol (23 ml) was refluxed for 1 h in a nitrogen atmosphere. After the usual work up, there was obtained 3.4 g of *Michael*-adduct. The crude material was refluxed for 30 min in a mixture of acetic acid (19.5 ml) and concentrated hydrochloric acid (29.5 ml). The resulting solution was taken up in water, saturated with sodium chloride and extracted with ethyl acetate. The organic layer was washed with sodium hydrogencarbonate and water, when evaporation left 1.55 g (79%) of crude diketone **10**. A sample purified by distillation had b.p. 76–78°/0.01 Torr; $d_4^{20} = 0.9809$; $n_D^{20} = 1.4549$. – IR. (neat): ν = 1120, 1360, 1380, 1405, 1710, 1730 cm^{-1}. – MS.: $M^+ = 168$, base peak m/e 43. – NMR.: δ = 1.03 (3 H, *s*); 1.07 (3 H, *s*); 2.16 (3 H, *s*); 2.63 (4 H, *m*); 1.3–2.2 (3 H, *m*).

$C_{10}H_{16}O_2$ (168.23) Calc. C 71.39 H 9.59% Found C 71.25 H 9.64%

Diketone **10** has also been found to occur in *Burley* tobacco condensate[7]).

A solution of the crude diketone **10** (1.55 g, 90% pure, 8.30 mmol) and *p*-toluenesulfonic acid (40 mg) in 2-methyl-2-ethyl-dioxolan (5 ml) was set aside for 2 h at room temperature [10]. GLC. (5% Carbowax, 200°, 2.5 m column) then showed the solution to contain about 73% of the desired mono-acetal **11**. It was poured into an excess of sodium hydrogencarbonate solution, extracted with ether, and the organic layer was washed once with brine. The crude product obtained after usual work up was distilled (0.001 Torr): Fr. 1, b.p. 64–66°, 0.40 g; Fr. 2, b.p. 71–73°, 0.60 g. These combined fractions contained about 0.84 g (48%) of monoacetal **11**. – IR. (neat) of a sample purified by GLC.: ν = 1040, 1375, 1730 cm^{-1}. – NMR.: δ = 1.0 (3 H, *s*); 1.05 (3 H, *s*); 1.31 (3 H, *s*); 1.55–2.80 (7 H, *m*); 3.92 (4 H, *s*).

Sodium hydride (96 mg of a 50% slurry in mineral oil, 2 mmol) was placed into a small flask (nitrogen atmosphere) and washed twice with light petroleum. A solution of mono-acetal **11** (106 mg, 80% pure, 0.4 mmol) in anhydrous dioxan (1 ml) was then added, followed by pure ethyl formate (1.5 ml) [11]. The mixture was stirred at room temperature until the hydrogen evolution subsided (2 h $^1/_2$). After destroying any unreacted sodium hydride with anhydrous ethanol, a slight excess of ammonium chloride (with respect to the Na present) was added to the mixture which was partly evaporated under vacuum, exactly neutralized by adding several drops of acetic acid, and extracted with ethyl acetate. The organic layer was washed once with brine, evaporated to dryness in the cold and the residue taken up in 50% ethanol (3 ml). This solution was acidified to pH 2 by adding 3 drops of 10% hydrochloric acid, and set aside for 1 h $^1/_2$ at room temperature with occasional shaking. Ammonium acetate (500 mg) was then added and the mixture refluxed for 2 h. Usual work up (simple ethereal extraction) afforded 140 mg of a crude product which was directly oxidized by $NaNO_2$/AcOH [20]. There was finally obtained 50 mg of a mixture containing **D** and **10** (~3:1) together with an important amount of high boiling material [GLC. separation on 5% Carbowax, 200°, 2.5 m column; relative R_T = 1.0 (**10**) and 1.6 (**D**); the high boiling by-products were not eluted]. This mixture could be conveniently separated by chromatography on 20 parts of silica gel[8]), in the course of which **10** was eluted with ether and *3,6,6-trimethyl-5,6-dihydro-7*H*-2-pyrindin-7-one* (**D**) with ethyl acetate. The latter substance, further purified by sublimation at 70°/0.001 Torr (yield 10 mg or 4.8% with respect to 5,5-dimethyl-cyclopent-2-en-1-one), exhibited the properties already discussed above in the theoretical part.

$C_{11}H_{13}NO$ (175.23) Calc. C 75.40 H 7.48 N 7.99% Found C 75.34 H 7.51 N 8.11%

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63. Preparation and Some Properties of Maleimido Acids and Maleoyl Derivatives of Peptides

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(31. XII. 74)

Summary. N-Alkoxycarbonylmaleimides **3** have been prepared and used to convert amino acids to maleimido acids (**6–8**) in aqueous solution. The carboxyl group of maleimido acids can be activated for amide or peptide synthesis (*e.g.*, in the N-succinimidyl esters **10**); *t*-butyl-based protecting groups can be cleaved without damage to the maleimide moiety. Peptides carrying maleimide groups are accessible either from the maleimido acids (*e.g.*, **11b**, **15**) or by direct maleoylation (*e.g.*, **16b**). The maleoyl group can be cleaved off by successive mild alkaline and acid hydrolysis or by hydrazinolysis. The reactivity of maleimides toward thiol groups suggests the use of maleimido acids and maleoylpeptides for preparing a wide range of conjugates of biochemical interest.

All attempts to prepare maleoylamino acids (maleimido acids) reported in the literature so far [2] [3] have failed.

Maleylamino acids (3-carboxyacryloylamino acids, **1**, R′= H) are readily accessible by reaction of the amino acids with maleic anhydride (see [2], [3] and references given there) but the cyclisation of the maleamic acid to the maleimide grouping (**1** → **2**, R′= H) has proved difficult in the presence of the additional free carboxyl group. *Helferich & Wesemann* [3] have cyclised the maleamic acid **1** (R = Me, R′= Et) derived from D,L-alanine ethyl ester to the maleimido ester

[1]) Part of the Doctoral Dissertation of *O. Keller* [1].