violett. MS.: 400 (M^+, 21), 369 (12), 299 (3), 268 (5), 258 (2), 244 (4), 210 (3), 198 (3), 184 (6), 182 (4), 167 (4), 156 (100), 138 (5), 125 (3), 101 (4).

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176. A Chemical Study of *Burley* Tobacco Flavour (*Nicotiana tabacum* L.) I. Volatile to medium-volatile constituents (b.p. $\leqq 84°/0.001$ Torr

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(3. V. 72)

Summary. The fraction b.p. $\leqq 84°$ (0.001 Torr) from *Burley* tobacco condensate was carefully investigated using fractional distillation and preparative column and gas liquid chromatography aided by GLC/MS coupling. Among the 193 compounds thus separated and characterized by their spectral data, 81 were newly identified tobacco constituents.

Most of the compounds isolated in the course of this work display flavouring properties which make them highly suitable for improving the flavour and aroma of tobacco and tobacco smoke.

1. Introduction. – As is well known, the characteristic flavour components of dried tobacco leaves can be isolated either by direct extraction with a volatile solvent,

or by steam distillation. The chemical composition of the essential oil thus obtained in small amounts has, of course, already been thoroughly investigated in order to provide a better understanding of the physiological and organoleptic properties of the so-called 'herbe à Nicot'. While it seems obvious that most of these analytical studies were carried out in a more or less close relation to the problem of *artificially flavouring tobaccos*, such a 'commercial interest' does not minimize their scientific significance in any way.

It is not our purpose here to give any survey of the voluminous tobacco literature. However, the reader interested in the chemistry of tobacco will find all relevant information in the review by *Stedman* [1], in which 666 papers published prior to September 27, 1967, are mentioned, and also in the pertinent chapters of *Gildemeister* & *Hoffmann*'s [2] and of *Arctander*'s [3] books. Among the more recent papers are those of *Bailey et al.* [4], *Creasy* & *Saxby* [5], *Hajime Kaneko et al.* [6], *Neurath et al.* [7], *Grob* & *Voellmin* [8], *Harke et al.* [9], *Hoffmann* & *Mazzola* [10], *Enzell et al.* [11], *Demole* & *Berthet* [12], *Groenen* & *van Gemert* [13], *Miller* & *Stedman* [14], *Schumacher* & *Heckman* [15], and *Roberts* & *Rohde* [16][1a]). Finally, beside this scientific literature, there also exist a plethora of patents from tobacco companies. The total volume of all of these publications is impressive and highly suggestive of the great difficulties involved in the chemical study of tobacco flavour and, above all, of tobacco smoke which probably contains more than 2600 constituents [8].

In order to give a brief definition of the so-called *Burley tobacco*, particularly for non-specialists, we must recall that four major tobacco types are commonly used for the manufacture of cigarette products: *oriental* tobacco (*e.g.*, Turkish, Greek), *'flue-cured'* tobacco (Virginia), *Maryland* and *Burley* tobacco. Since each of these four types of tobacco displays very distinct organoleptic features (the oriental variety being richest in flavour), they are best used as *blends* for the manufacture of cigarettes. Thus, the flavour, aroma and quality of cigarette products depend upon the particular type of mixture of tobaccos employed. *Burley* tobacco, an initial report on the flavour of which is presented here, is one of the important raw materials for the cigarette industry.

2. Results and discussion. – The constituents isolated and identified from fractions B1, B2-PA and B2-PN (b.p. not exceeding 84°/0.001 Torr; see Scheme 1) of *Burley* tobacco condensate are listed in the Table given below, according to their functional groups and molecular weights. These compounds were all separated as shown in Schemes 1 to 13, by applying the general procedure described in the experimental chapter entitled 'Method'. In this Table we give from left to right: 1. The chemical names of the compounds. 2. The numbering of the corresponding structural formulae. 3. The No. of the related Schemes (2 to 13) in which each preceding structural formula has to be found. 4. The molecular weights. 5. The substances which, to the best of our knowledge, should be considered as new tobacco constituents, and which are marked by a cross. 6. The literature (corresponding either to a previously published review or paper mentioning the known constituents of tobacco leaves or tobacco smoke, or to a convenient synthetic method for certain new constituents).

[1a]) We thank Dr. *Roberts* and Dr. *Rhode* for having made their manuscript available before publication.

Volatile to medium-volatile constituents of Burley *tobacco condensate*
(Identified from fractions B1, B2-PA and B2-PN, Scheme 1)

1. *Aliphatic, Alicyclic, and Aromatic compounds*

1a. *Free Carboxylic Acids*	Formula	Schemes	M^+	New in tobacco	References
Formic acid	1	3	46		[1]
Acetic acid	2	3	60		[1]
Propionic acid	3	3	74		[1]
Tiglic acid	4	3	100	+	
Pentanoic acid	5	3	102		[1]
3-Methylbutyric acid	6	2, 3	102		[1]
2-Methylbutyric acid	7	3	102		[1]
2-Hexenoic acid (*trans* ?)[1]	8	3	114		[5]
Hexanoic acid	9	3	116		[1]
Benzoic acid	10	2, 3	122		[1]
2-Heptenoic acid (*trans* ?)[1]	11	3	128		[5]
5-Methylhex-2-en-oic acid (*trans*)	12	3	128		[16]
Heptanoic acid	13	3	130		[1]
4-Methylhexanoic acid	14	3	130	+	
Octanoic acid	15	3	144		[1]
Dimethyl-maleic (or -fumaric) acid[1][2]	16	3	144	+	
Nonanoic acid	17	3	158		[1]
Ethylmethyl-maleic (or -fumaric) acid[1][2]	18	3	158	+	
Decanoic acid	19	3	172		[1]
Mixture of C_6–C_8 acids, unsaturated and/or branched					
1b. *Alcohols*					
2-Methyl-1-butanol	20	2	88	+	
3-Methyl-1-butanol	21	2	88	+	
1-Pentanol	22	2, 12	88	+	
Hex-3-en-1-ol (*cis*)	23	2, 10	100		[16]
1-Hexanol	24	2, 11	102	+	
Benzyl alcohol	25	2, 3, 9–12	108		[1]
2-Methylpentane-2,4-diol	26	13	118	+	
1-Phenylethanol	27	10, 11	122	+	
2-Phenylethanol	28	3, 9–12	122		[1]
6-Methylhept-5-en-2-ol	29	2, 10, 11	128		[16]
1-Octen-3-ol	30	9	128	+	
3-Octanol	31	9	130	+	
1-(*p*-Tolyl)-ethanol	32	11	136	+	
2,3-Octanediol	33	13	146	+	
Linalool	34	8, 9	154		[1]
α-Terpineol	35	10, 11	154	+	
Geraniol	36	10, 11	154	+	
1,3,3-Trimethyl-cyclohexane-1,2-diol[1]	37	12, 13	158	+	
4,8-Dimethyl-nona-3,7-dien-1-ol	38	10, 11	168	+	
Linalool oxide (2 stereomers)	39	11	170	+	
Solanol	40	9, 10	196		[16]
6,10-Dimethyl-undecan-2-ol	41	9	200	+	

[1] Stereochemistry undetermined.

[2] These diacids are possibly artifacts arising from the partial hydrolysis of the corresponding anhydrides during treatment with 10% Na_2CO_3 (Scheme 1).

1c. *Aldehydes*	Formula	Schemes	M^+	New in Tobacco	References
Benzaldehyde	42	6, 7, 8	106		[1]
2,4-Heptadienal (*trans/trans*)	43	8	110	+	
Phenylacetaldehyde	44	6, 8–10	120	+	
o-Methylbenzaldehyde	45	6	120	+	
o-Hydroxybenzaldehyde	46	6	122		[1]
Cinnamaldehyde	47	8	132	+	
α-Phenylcrotonaldehyde	48	8, 9	146	+	[17]
p-Isopropylbenzaldehyde	49	9	148	+	
β-Cyclocitral	50	7	152		[11b]
Decanal	51	5	156		[11b]
2,6,6-Trimethyl-4-oxo-cyclohex-2-enylidene-acetaldehyde[1])	52	9, 11	178		[16]
Tridecanal	53	5	198	+	
Pentadecanal	54	5	226		[11b]
1d. *Amides*					
N-(3-Methylbutyl)-formamide	55	13	115	+	
N-(3-Methylbutyl)-acetamide	56	13	129	+	
1e. *Anhydrides*					
Dimethylmaleic anhydride	57	2, 3	126		[16]
Ethylmethylmaleic anhydride	58	2, 3	140		[16]
1f. *Ketols*					
2,6,6-Trimethyl-4-hydroxy-cyclohex-2-en-1-one	59	12	154	+	
3,3,5-Trimethyl-4-hydroxy-cyclohexan-1-one[1])	60	12	156	+	[18]
2,2,6-Trimethyl-4-hydroxy-cyclohexan-1-one[1])	61	13	156		[16]
5-(1-Hydroxy-1-methyl-ethyl)-2-methyl-cyclohex-2-en-1-one	62	6	168	+	
2,2,6,7-Tetramethyl-7-hydroxy-bicyclo[4.3.0]non-1(9)-en-8-one[1])	63	11	208		[16]
1g. *Ketones*					
2,3-Dimethyl-cyclopent-2-en-1-one	64	11	110		[19]
3-Methyl-cyclohex-2-en-1-one	65	11	110	+	
4-(Methylthio)-2-butanone	66	9	118	+	[20]
Acetophenone	67	2, 7–9	120		[19]
3,5-Octadien-2-one (*trans/trans*)	68	8, 9	124	+	
6-Methyl-hepta-3,5-dien-2-one (*trans*)	69	2, 9–11	124		[11b]
6-Methyl-hept-5-en-2-one	70	2, 8, 9	126		[11b]
Phenylacetone	71	8	134	+	
p-Methylacetophenone	72	8, 9	134		[1]
Isophorone	73	2, 6, 9–11	138		[7b]
6-Methyl-2,5-heptanedione	74	2, 10–12	142		[16]
2,3-Octanedione	75	9	142	+	
3,5,5-Trimethyl-4-methylene-cyclohex-2-en-1-one	76	2, 9, 10	150		[16]
2,6,6-Trimethyl-cyclohex-2-ene-1,4-dione	77	2, 8–10	152		[7b]

	Formula	Schemes	M^+	New in tobacco	References
3-Acetyl-isopropenyl-cyclopentane[1])	78	2, 8, 9	152		[16]
Piperitone	79	9	152		[11b]
2,2,6-Trimethyl-cyclohexane-1,4-dione	80	9–11	154		[16]
2-Acetonyl-4-methyl-tetrahydropyran (*cis*)	81	11	156		[16]
2,6,6-Trimethyl-2,3-epoxy-cyclohexane-1,4-dione	82	2, 8, 9	168	+	
2-Undecanone	83	8	170	+	
2,5,8-Nonanetrione	84	8	170	+	
2-Acetonyl-3-isopropyl-tetrahydrofuran (*trans*)	85	11, 12	170	+	[16]
1,5,5-Trimethyl-9-oxa-bicyclo[4.3.0]non-6-en-3-one	86	10, 11	180		[16]
1,5,5-Trimethyl-9-oxa-bicyclo[4.3.0]nonan-3-one[1])	87	11–13	182		[16]
4-(1,3-Butadienyl)-3,5,5-trimethyl-cyclohex-2-en-1-one (*trans*)	88	8–10	190		[16]
4-(2-Butenylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one (4 stereomers)	89	8–11	190		[16]
β-Damascenone	90	7, 8	190		[12]
4-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-but-3-en-2-one	91	8	190	+	
β-Ionone	92	8, 9	192		[12]
β-Damascone (*trans*)	93	8	192		[12]
6,10-Dimethyl-undeca-3,5,9-trien-2-one (*trans*/*trans*)	94	8	192		[11b]
Isosolanone	95	7	194	+	[21]
Solanone	96	2, 6–9	194		[21]
6,10-Dimethyl-undeca-5,9-dien-2-one	97	7–9	194		[1]
Norsolanedione	98	6, 7, 11, 12	196		[19]
6,10-Dimethyl-2-undecanone	99	6–8	198		[12]
2-Acetonyl-3-isopropyl-6-methyl-tetrahydropyran (2 stereomers)	100	9–11	198		[16]
4-(2,6,6-Trimethyl-1,2-epoxy-cyclohexyl)-but-3-en-2-one	101	8, 9, 11	208		[12]
Teaspirone	102	11	208	+	[22]
2,6,6,10-Tetramethyl-1-oxa-spiro[4.5]dec-2-en-8-one	103	11, 12	208	+	
1,3,7,7-Tetramethyl-2-oxa-bicyclo[4.4.0]dec-5-en-9-one[1])	104	9–11	208	+	[23]
1,3,7,7-Tetramethyl-2-oxa-bicyclo[4.4.0]decan-9-one (2 stereomers)	105	10, 11	210		[16]
1h. *Esters*					
Methyl benzoate	106	5, 6, 8	136	+	
Benzyl formate	107	6	136	+	
2-Phenylethyl formate	108	6	150	+	
2-Phenylethyl acetate	109	7, 8	164		[1]
Methyl dodecanoate	110	5	214	+	
2-Phenylethyl hexanoate	111	11	220	+	
Ethyl phthalate	112	8	222	+	
Methyl tetradecanoate	113	5	242		[1]
Butyl phthalate	114	8	278		[1]

1i. *Hydrocarbons*	For-mula	Schemes	M^+	New in tobacco	Refer-ences
Naphthalene	115	4	128		[1]
H. $C_{12}H_{24}$	116	5	168		[1]
H. $C_{13}H_{26}$	117	5	182		[1]
H. $C_{13}H_{28}$	118	4	184		[1]
H. $C_{14}H_{26}$	119	5	194	+	
H. $C_{14}H_{30}$	120	4	198		[1]
Caryophyllene	121	4	204	+	
Other sesquiterpene hydrocarbons		4	204		
H. $C_{16}H_{34}$	122	4	226		[1]
H. $C_{18}H_{38}$	123	4	254		[1]
H. $C_{19}H_{38}$	124	4	266		[1]
Neophytadiene	125	4	278		[1]
1j. *Lactones*					
2-Methyl-but-2-en-4-olide	126	2, 10, 11	98	+	
3-Methyl-but-2-en-4-olide	127	12	98	+	
3-Methyl-pent-2-en-5-olide	128	12, 13	112	+	[24a]
4,4-Dimethyl-but-2-en-4-olide	129	2, 10, 11	112	+	
4-Ethyl-but-2-en-4-olide	130	10, 11	112	+	
4-Ethyl-butan-4-olide	131	13	114		[16]
3-Isopropyl-but-2-en-4-olide	132	10	126	+	[24b]
4-Ethyl-4-methyl-butan-4-olide	133	10	128		[16]
2-Isopropyl-butan-4-olide	134	9	128		[16]
3-Isopropyl-pent-2-en-5-olide	135	11	140	+	[24a]
3-Isopropyl-pentan-5-olide	136	10, 11	142		[16]
Dihydro-actinidiolide	137	9–11	180		[4]
Bovolide	138	7, 8	180	+	[24b]
Dihydro-bovolide	139	8, 9	182		[6b]
1k. *Phenols and Phenolic Ethers*					
Phenol	140	8, 9	94		[1]
o-Cresol	141	8	108		[1]
p-Cresol	142	8, 9	108		[1]
p-Vinyl-phenol	143	8	120	(+)[3]	[8]
o-Methoxy-phenol	144	2, 6–9	124		[1]
o-Methoxy-*p*-vinyl-phenol	145	6, 7	150	+	
o-Acetyl-*p*-cresol	146	6	150	+	
Methyl salicylate	147	5	152		[1]
5-Isopropenyl-2-methyl-anisole	148	4	162	+	[25]
2. *Heterocyclic compounds*					
2a. *Furans*					
2-Furaldehyde	149	2, 8	96		[1]
Furfuryl alcohol	150	2, 11	98		[1]
5-Methyl-2-furaldehyde	151	2, 8, 9	110		[1]

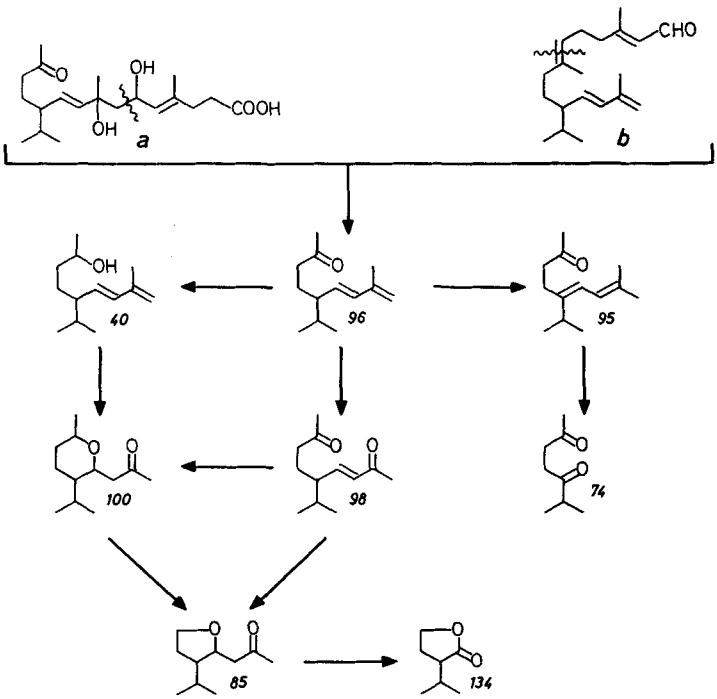
[3]) Mentioned in the literature only as a general structure including all positional isomers.

	For-mula	Schemes	M^+	New in tobacco	Refer-ences
2-Acetylfuran	152	2, 9, 11	110		[1]
2-Acetyl-5-methyl-furan	153	9, 10	124		[7b]
5-Acetyl-2-furaldehyde	154	8	138	+	
3-Phenylfuran	155	4	144	+	
2b. *Pyrazines*					
Pyrazine	156	2	80		[1]
2-Methylpyrazine	157	2	94		[1]
2,3-Dimethylpyrazine	158	2, 13	108		[7a]
2,6-Dimethylpyrazine	159	2, 13	108		[1]
2-Isopropenylpyrazine	160	12	120	+	
2-Vinyl-6-methyl-pyrazine	161	2, 11, 12	120	+	
2-Acetylpyrazine	162	2, 11, 12	122		[16]
2-Ethyl-5-methyl-pyrazine	163	2, 13	122		[7b]
2-Ethyl-6-methyl-pyrazine	164	13	122		[7a]
2,3,5-Trimethylpyrazine	165	2, 13	122		[7a]
2-Acetyl-3-methyl-pyrazine	166	11	136	+	[26]
2-Acetyl-6-methyl-pyrazine	167	2, 11, 12	136		[16]
2-Ethyl-3,6-dimethyl-pyrazine	168	13	136	(+)[3]	[7a]
2c. *Pyridines*					
3-Cyanopyridine	169	12	104		[7a]
3-Formylpyridine	170	13	107		[1]
3-Propenylpyridine	171	12, 13	119	+	
2-Acetylpyridine	172	10	121	+	
3-Acetylpyridine	173	13	121		[1]
3-Propionylpyridine	174	13	135		[1]
5-Isopropyl-2-methyl-pyridine	175	13	135		[7b, 27]
3-Phenylpyridine	176	11, 12	155		[7a]
Nicotyrine	177	12, 13	158		[1]
2d. *Pyrroles*					
2-Formylpyrrole	178	10, 11	95		[28]
2-Acetylpyrrole	179	11	109		[1]
2-Formyl-1-methyl-pyrrole	180	2, 8, 9, 11	109		[16]
Indole	181	4, 5	117		[1]
2-Acetyl-5-methyl-pyrrole	182	11	123		[16]
3-Acetyl-1-methyl-pyrrole	183	5	123	+	
1-(3-Methylbutyl)-2-formyl-pyrrole	184	6, 7, 8	165	+	
2e. *Thiophenes*					
2-Formyl-3-methyl-thiophene	185	7	126	+	
2-Formyl-5-methyl-thiophene	186	8	126	+	
2-Acetylthiophene	187	8	126	+	

3. *Miscellaneous*	For-mula	Schemes	M^+	New in tobacco	Refer-ences
1-Acetylimidazole	188	2	110	+	
α,β-Epoxy-styrene	189	2	120	+	
Quinoline	190	12	129		[1]
Benzothiazole	191	8, 9	135	+	
Methylethylmaleimide	192	12	139	+	
4-Methylquinoline	193	12	143	(+)[3]	[7a]

The 193 compounds listed in the above Table make up about 87% of the total weight of the starting fractions (B1, B2-PA and B2-PN, see Scheme 1). As a rule, these tobacco constituents were identified by their mass spectra, combined in many instances with IR., NMR. and UV. data. Indeed, mass spectrometry alone would not have enabled elucidation of the relatively complex structures encountered, especially in the terpene series. Finally, in most cases, the correctness of the assigned structures was demonstrated by directly comparing the spectra of the natural compounds with those of authentic, synthetic samples[4]).

The present investigation, which covered the range of molecular weights between 46 and 278, resulted in the identification of 81 new constituents of tobacco flavour.

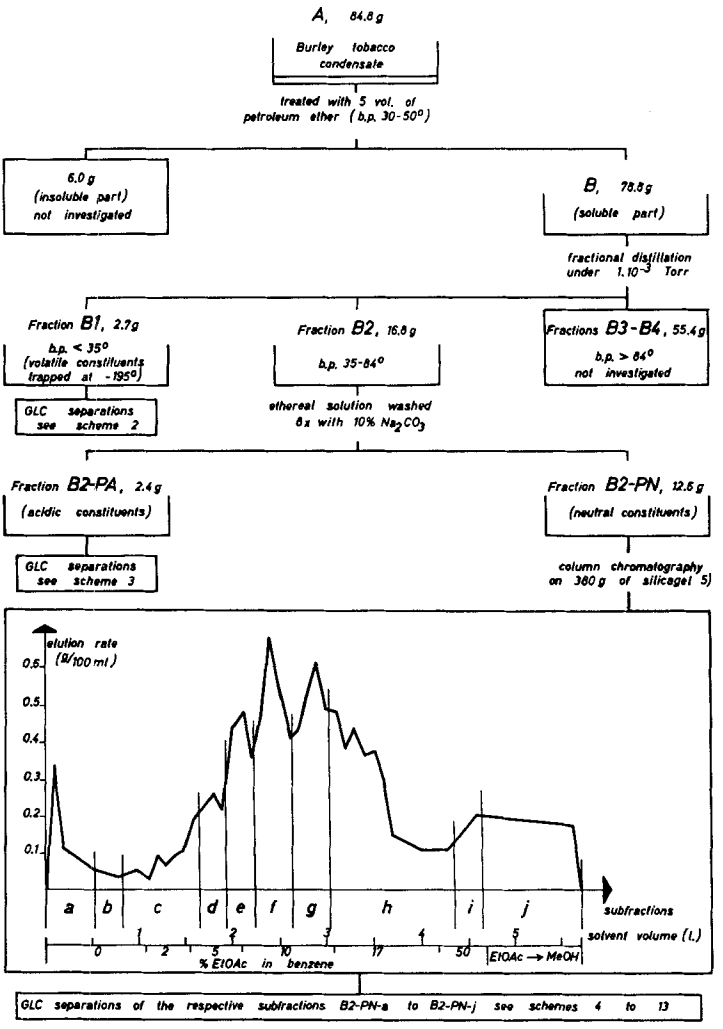


[4]) Some synthetic work will be published later, as well as a full description of the characteristics of the substances investigated.

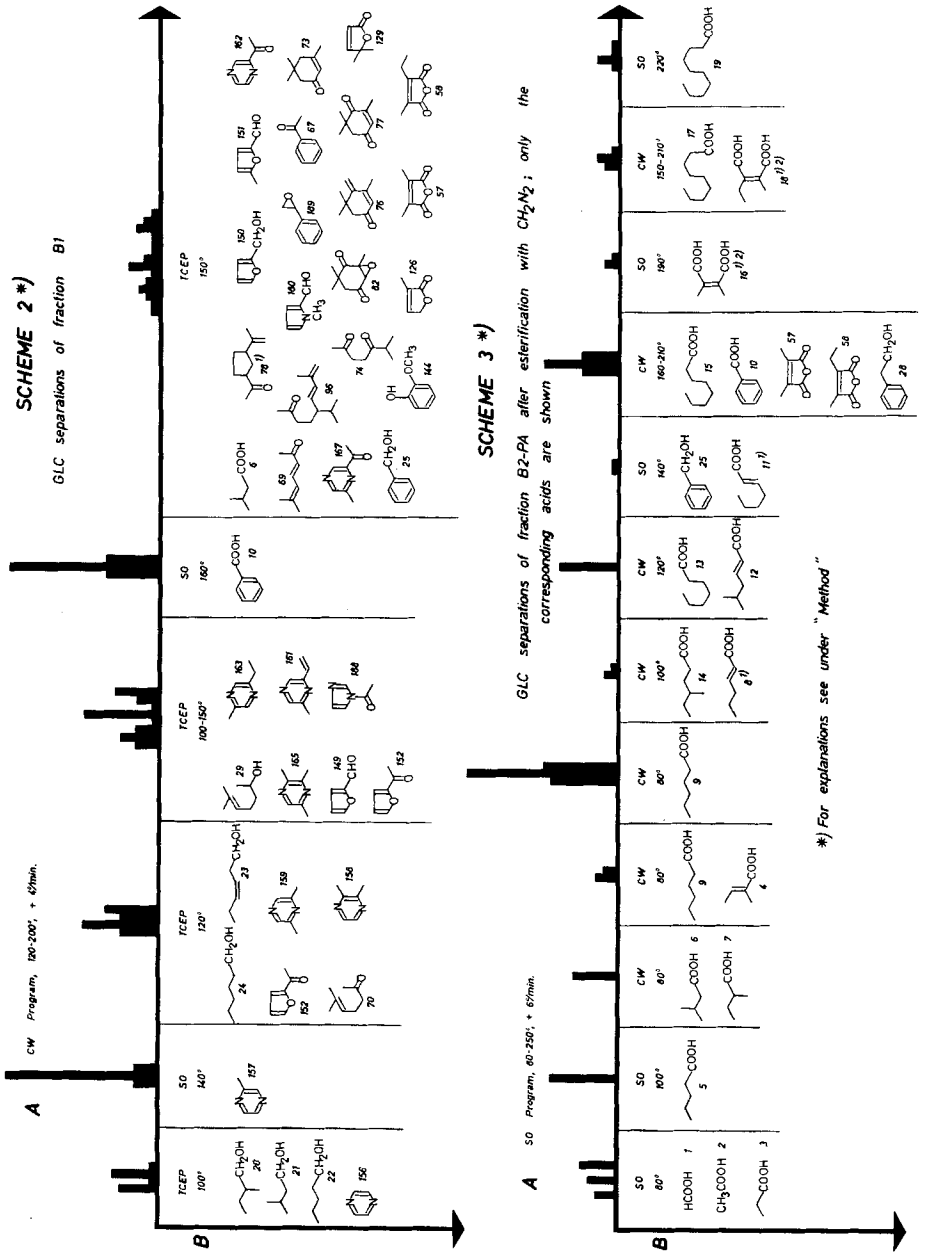
Moreover, and not surprisingly, our results strongly suggest that a large number of flavouring constituents of the *Burley* tobacco condensate are closely related *metabolites* or degradation products, which probably arise from the numerous chemical and enzymatic reactions favoured by the aerobic treatment of the tobacco leaves. Highly significant in this respect is the fact that many of the constituents listed in the above Table can be grouped into several well-defined 'families'. One of these families would include all the regular terpenoids belonging to the C_{13} (63, 88–93,

SCHEME 1 *)

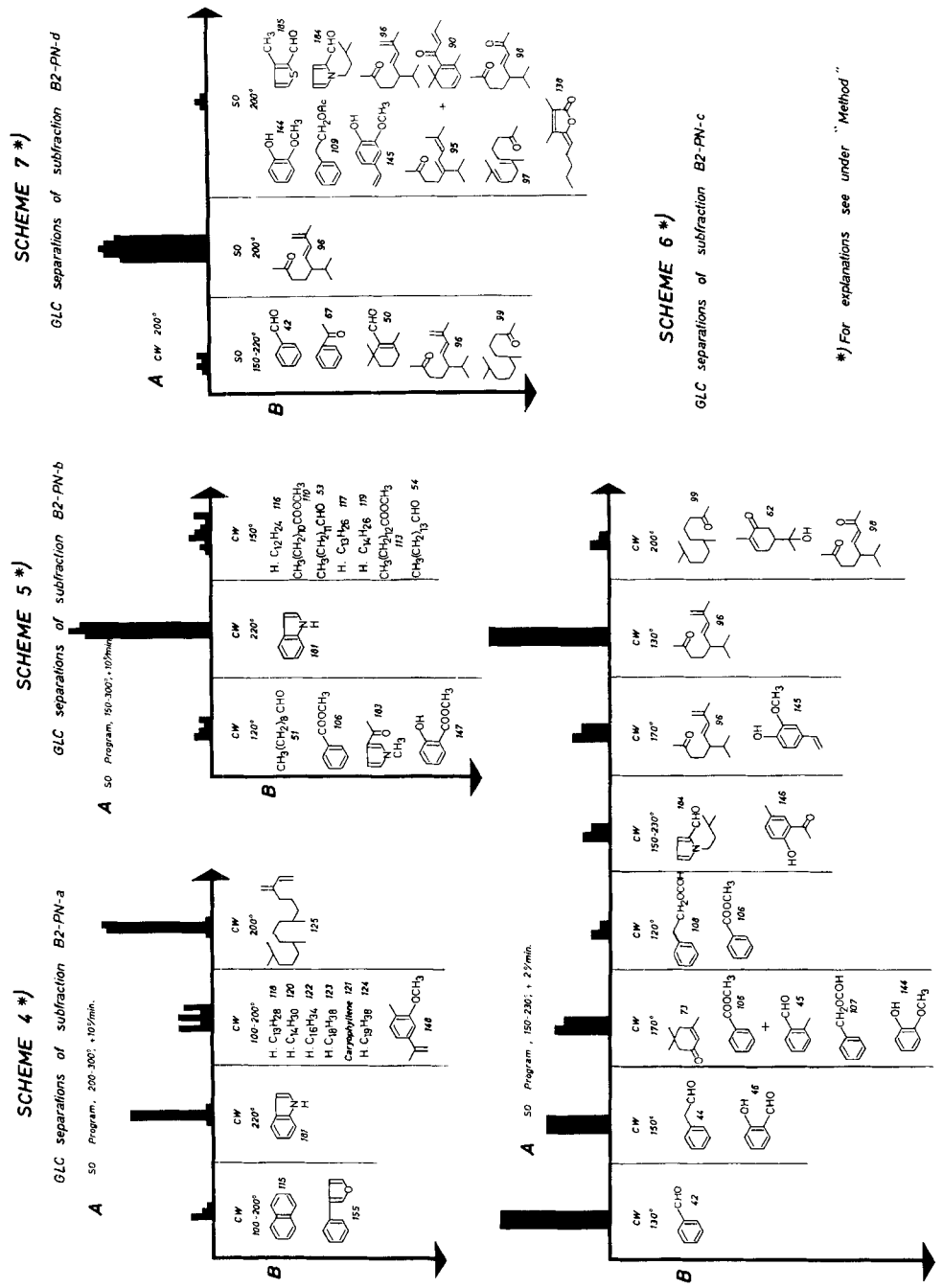
Preliminary steps of Burley tobacco condensate analysis



*) For explanations see under "Method"

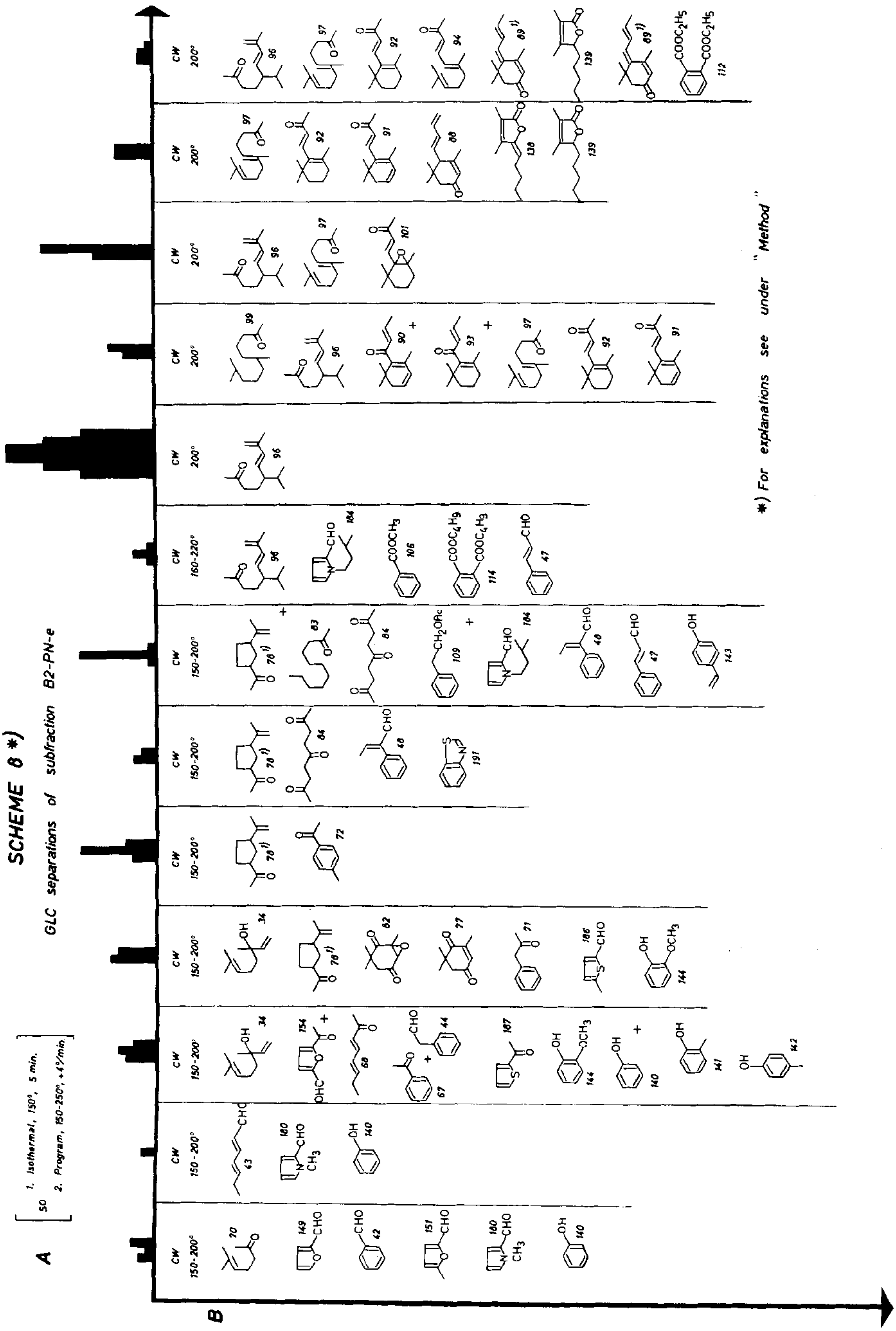


101–105), C_{11} (52, 86, 87, 137) and C_9 (37, 59–61, 73, 77, 80, 82) series, which apparently correspond to three successive steps of an oxidative degradation of higher terpenes, presumably of carotenoids [29] [30]. Such a process would be consistent with the fact that some of these C_{9-13} terpenoids are rather common natural substances (for instance several of the C_9 representatives also occur in saffron [31]). A second 'family'

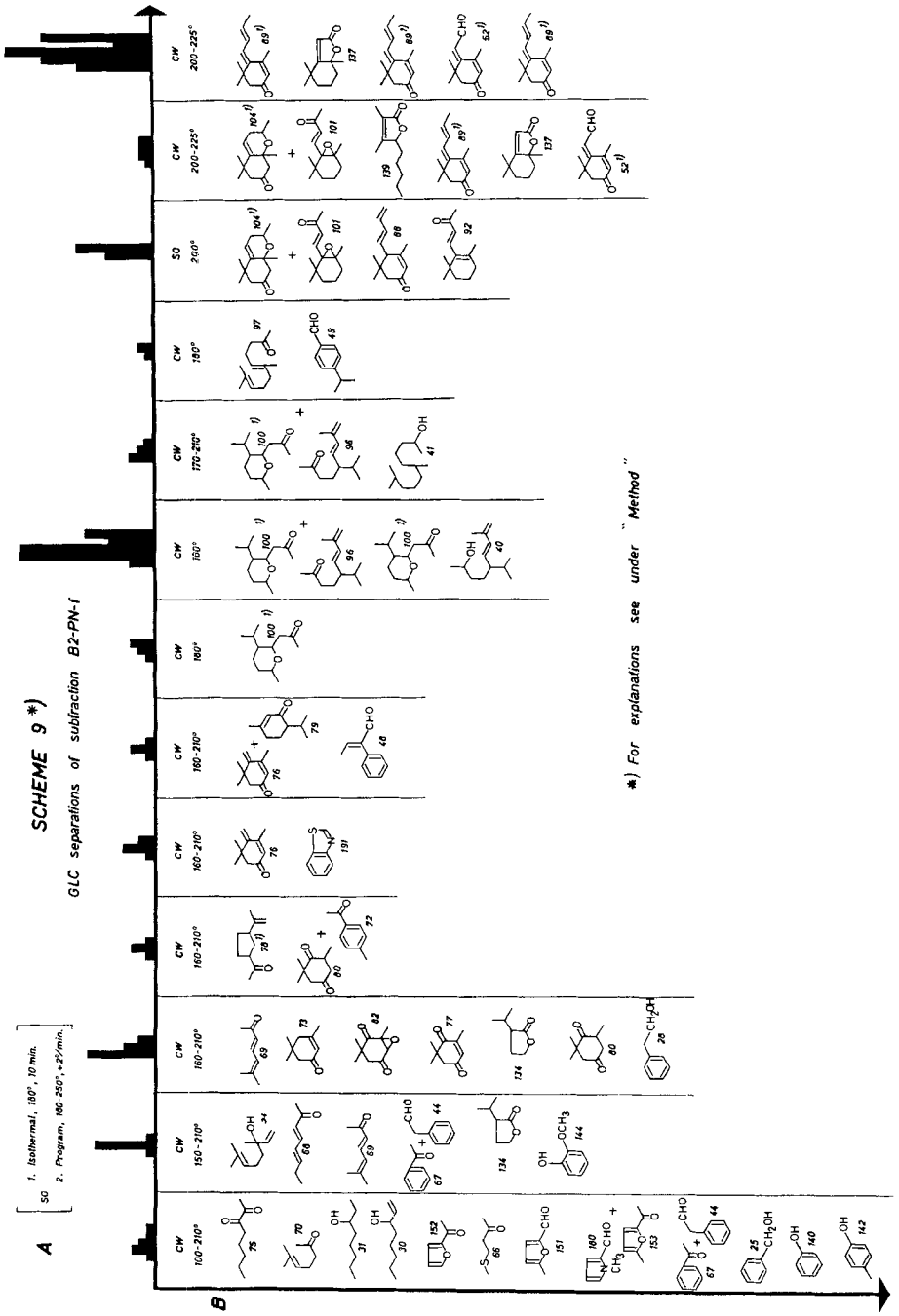

SCHEME 4 *)
GLC separations of subfraction B2-PN-a
SCHEME 5 *)
GLC separations of subfraction B2-PN-b
SCHEME 6 *)
GLC separations of subfraction B2-PN-c
SCHEME 7 *)
GLC separations of subfraction B2-PN-d
*) For explanations see under "Method"

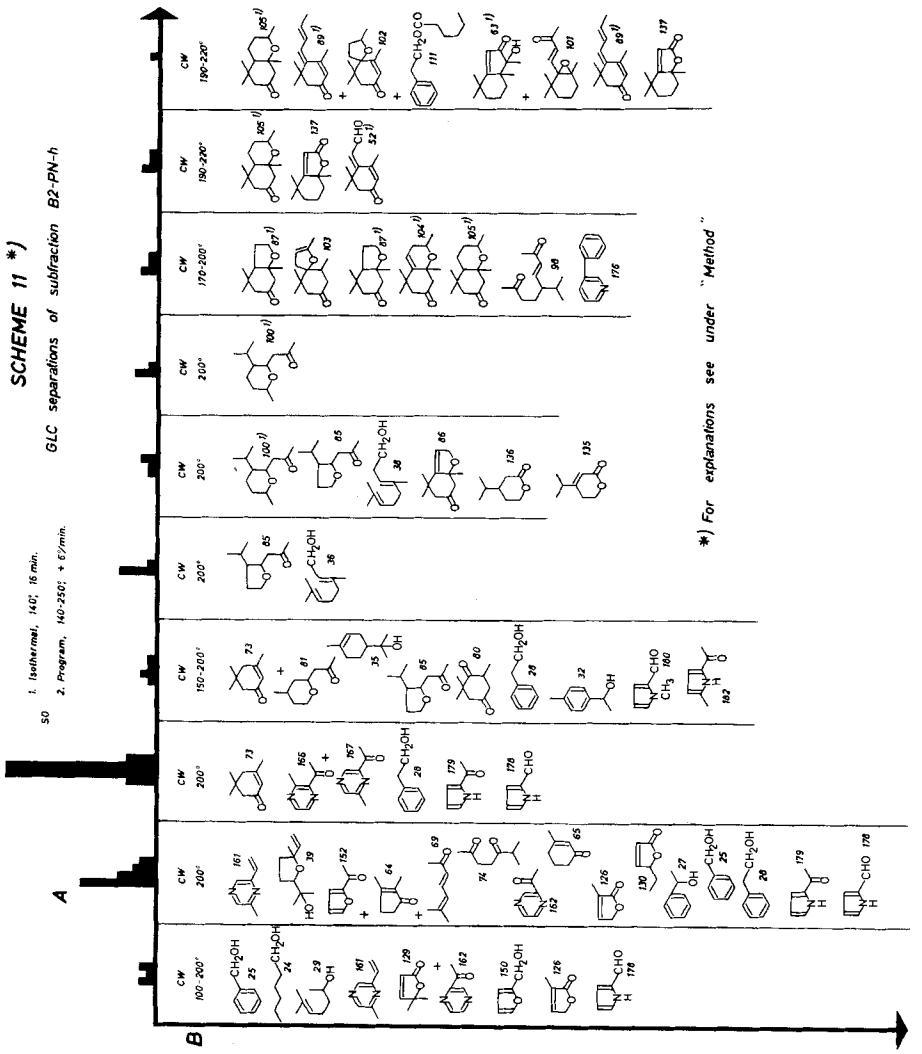
SCHEME 8 *)

GLC separations of subfraction B2-PN-e



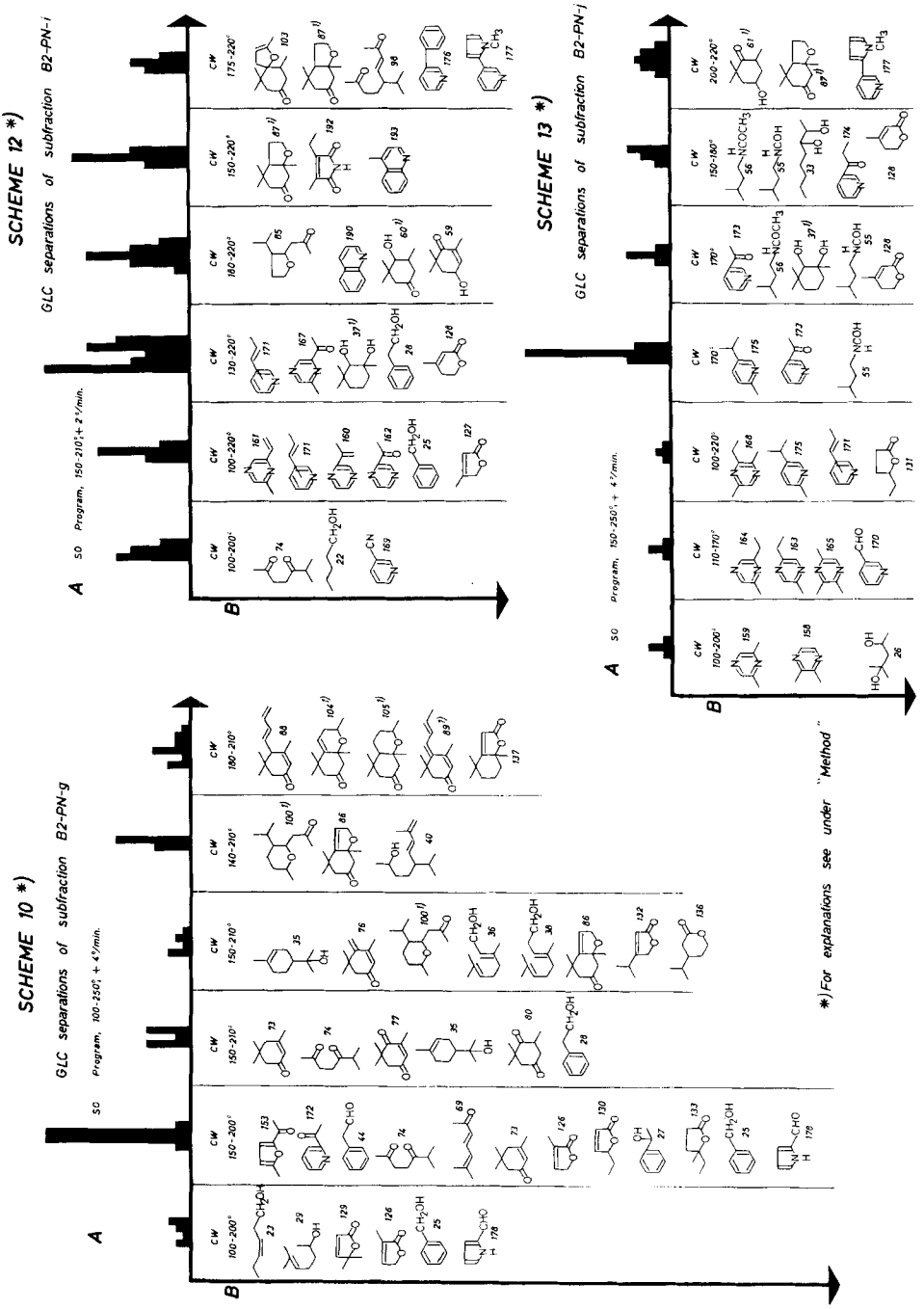
*) For explanations see under "Method"


SCHEME 9 *)
GLC separations of subfraction B2-PN-f
A
SG
1. Isothermal, 180°, 10 min.
2. Program, 180–250°, +2°/min.
B
CW 100–210°
CW 150–210°
CW 160–210°
CW 160–210°
CW 160–210°
CW 160–210°
CW 160°
CW 160°
CW 170–210°
CW 180°
SG 200°
CW 200–225°
CW 200–225°
*) For explanations see under "Method"



of metabolites, which appears to be more typical of tobacco, includes *solanone* (96) and its 'derivatives' 40, 74, 85, 95, 98, 100, 134. All of these irregular terpenoids and related compounds may result from the degradation of the diterpene precursors *a* [32] and *b* [33], which could themselves originate from the α- and β-duva-4,8,13-triene-1,3-diols [34] by ring cleavage. Finally, most of the 3-substituted pyridines mentioned in the above Table should also be considered as a further tobacco metabolite 'family' related to nicotine.

Generally, the exceedingly complex and heteroclite chemical composition of the *Burley* tobacco condensate makes it difficult to evaluate its constituents individually, according to their flavouring importance. In this initial stage of our research work, we assume that all compounds listed in the above Table must participate in some way


SCHEME 10 *)
GLC separations of subfraction B2-PN-g
A
SO
Program, 100-250° + 4°/min.
B
CW 100-200°
CW 150-200°
CW 150-210°
CW 140-210°
CW 150-210°
CW 180-210°
*) For explanations see under "Method".
SCHEME 12 *)
GLC separations of subfraction B2-PN-i
A
SO
Program, 150-210° + 2°/min.
B
CW 100-200°
CW 100-220°
CW 130-220°
CW 160-220°
CW 150-220°
CW 175-220°
SCHEME 13 *)
GLC separations of subfraction B2-PN-j
A
SO
Program, 150-250° + 4°/min.
B
CW 100-200°
CW 110-170°
CW 100-220°
CW 170°
CW 150-160°
CW 200-220°

in the *Burley* tobacco flavour and, consequently, should be tested in artificial reconstructions of this flavour.

3. Method. – The starting material for this work was a *Burley* tobacco of a grade commonly employed for the manufacture of high quality cigarette products. After adjustment to pH 4, the aqueous condensate resulting from the treatment of this tobacco with dry steam at 120° was extracted twice with chloroform, and the combined extracts were cautiously evaporated to dryness. The remaining product represented the so-called *Burley tobacco condensate*, obtained in a yield of approximately 0.03% by weight of starting tobacco. This essential oil appears to be substantially free of nicotine and displays excellent organoleptic properties.

The general path of the preliminary fractionation of the *Burley* tobacco condensate is illustrated in Scheme 1. It will be noticed that the use of gas liquid chromatography (GLC) *alone* was sufficient to complete the study of the most volatile, B1 fraction. However, such a simple technique could not be applied to fraction B2 (b.p. 35–84°/0.001 Torr), which was a more complex mixture. In this case, it was necessary to remove the acidic constituents first (B2-PA), and then to subject the remaining neutral part of the fraction to a chromatographic separation on silicagel[5]). This procedure allowed us finally to obtain 10 subfractions (B2-PN-a to -j) of relatively simple composition, suitable for the next GLC study.

The main steps of the GLC separations of the above mentioned fractions and subfractions (B1, B2-PA, B2-PN-a to -j) are shown in Schemes 2 to 13. All of these separations were made using a gas chromatograph[6]) fitted with conventional, analytical columns [(0.4–0.6) × (250–500) cm]. As a rule, whenever the mass spectra alone did not permit unequivocal identification of the separated substances, we trapped 5 to 500 mg of these in order to measure the corresponding IR., NMR., or UV. spectra, or to obtain further useful information[7]). To perform these numerous (about 5000 to 6000) GLC separations we usually combined several well-contrasted types of stationary phases: silicone oil (SO)[8]), carbowax (CW)[9]), and TCEP[10])[11]).

Each fraction or subfraction of the *Burley* tobacco condensate was first subjected to a 'semi-preparative' separation, most often on SO columns, symbolized in Schemes 2 to 13 by arrow *A*. Above this arrow, the peaks of these preliminary chromatograms in their proper ratios have been drawn and the experimental parameters used in each case are given. The 'peaks' trapped in the course of these separations were then re-injected onto another stationary phase such as *CW*, and this resulted in the final chromatograms symbolized by arrow *B* in our Schemes. In rows parallel to this arrow, the formulae of the compounds isolated and identified in each of these sub-separations[12]) are indicated according to the order of elution (downwards and occasionally from left to right), as are the experimental conditions used. The sign + separating certain formulae means that the corresponding substances showed nearly identical retention times and were not separated at this stage[12]).

During these separations a large number of additional constituents were also isolated which escaped identification, and which consequently do not appear in the Schemes 2 to 13. However, on the whole, these unknown substances made up to only 13% by weight of the investigated fraction of *Burley* tobacco condensate. Most of them occurred in trace amounts and were characterized by

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[5]) 'Kieselgel 0.05–0.2 mm für die Säulenchromatographie' (*Merck AG*).

[6]) *'Aerograph'*, Model 1820–3 (*Varian Aerograph AG*).

[7]) The spectra were run in our specialized laboratories under the supervision of Drs. *B. Willhalm* & *F. Gautschi*, on the following instruments: mass spectrometer *Atlas* CH4 1V 58 (Atlas-Werke AG); double-beam IR. spectrometer *Perkin-Elmer* 125; UV. spectrometers CF4 N.I. (*Optica*) and *Beckman*; NMR. spectrometer *Varian* A-60.

[8]) *'Embaphase'* (*May & Baker* Ltd).

[9]) *'Carbowax 20 M'* (*Varian* Acrograph AG).

[10]) Tris-1, 2, 3-(2-cyano-ethoxy)-propane or *'Fractonitril III'* (*Merck AG*).

[11]) The columns used were packed with 5–15% of these stationary phases on *'Chromosorb W'* (*Johns-Manville*).

[12]) In many instances, the substances isolated in the course of the sub-separations *B* were further purified by GLC on SO. Incidentally, this last refinement allowed us to complete the few defective separations which subsisted.

their mass spectrum only. We are in the course of investigating some of these unknown, possibly interesting flavour components.

The GLC/MS coupling technique was used throughout this work only for the limited purpose of *monitoring* the accuracy and the working efficiency of the preparative separations carried out on fractions and subfractions B1, B2-PA, and B2-PN-a to -j (Scheme 1).

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