

COLLABORATIVE PROJECT WITH SVENSKA TOBAKS A.B.

VISIT TO SVENSKA TOBAKS A.B., STOCKHOLM

18TH - 19TH JANUARY, 1982

Present: Prof. C.R. Enzell - Svenska Tobaks,
Dr. I. Wahlberg - Research Department,
Mr. R. Arndt - Stockholm.

Mr. B. Kimland - Svenska Tobaks, Product
Mr. B. Lindberg - Development Department, Malmö

Dr. R.A. Crellin - B.A.T., GR & DC
Dr. R.N. Penn - Southampton.

Purpose

Dr. Wahlberg and Mr. Arndt visited Southampton on 25th/26th June 1981 (see visit note dated 6th July 1981). At that time, considerable discrepancy existed between the sensory testing results from the two Companies on the limited number of samples available. Since then, a further number of samples have been generated and considerable sensory work has been carried out. The meeting was held to compare the more recent sensory data, examine chromatographic and biological analytical results, consider future gum production requirements and plan the next phase of experimental work.

The programme for the meeting is attached to this note: also attached is a description of the samples coded S1 - S25 referred to throughout this note.

This note is divided into the following sections:

1. Description of Svenska Tobaks Product Development activities (Kimland)
2. Intercomparison of Sensory Results (Lindberg/Penn).

103472788

3. Examination of Gas Chromatographic Results (Wahlberg/Arndt)
4. Examination of Biological Results (Pettersson - University of Stockholm).
5. Future Green Leaf Extract Requirements (Crellin)
6. Future Experimental Plans.

1. SVENSKA TOBAKS PRODUCT DEVELOPMENT DEPARTMENT

Product Development has been in existence now for 20 years and is centred at Gothenburg (snuff and chewing tobaccos) and Malmö (cigarettes and cigars). The brief is to develop new products and improve existing products. The Department consists of 20 people working in small groups. The staff have a good knowledge of blending and buying and travel with Leaf Department staff on buying visits. Factory knowledge is required since Product Development are responsible for new products until Production Department is completely ready to assume responsibility.

5 of the 20 people are concerned with flavours and have contact with Flavour Houses. Lindberg is responsible for the Sensory Panel Group and the sensory panels are:

- a) External Panel - 10 persons selected and trained.
- b) Internal Panel - works with a wide range of descriptors and scores intensities.
- c) Expert Panel - involves senior staff responsible for the project concerned.

The Product Development Department tries to apply the findings of the Research Department and the B.A.T. Project is one of the major areas of application at present.

2. RESULTS OF SENSORY TESTING

A. Malmö Product Development Department results

To date the above panel have only examined fractions S1-13. Their panel method is based on that of an open, paired-comparison

103472789

type using descriptors and magnitude estimation. Levels of application on their Test cigarette (PU20) were 250g and 750g per 100Kg tobacco, contrasting with the GR & DC panel which examined all fractions at 300g/100Kg tobacco.

Each of the samples was described in terms of amplitude of tobacco aroma, degree of irritation, aftertaste and then ranked in sub-groups according to order of acceptability.

Although the ranking of S6 within its sub group was not high, it was found by the Malmö panel to be of particular interest in its effect on enhancing the Burley character of the test cigarette. A profile of this fraction was obtained, against the control, using a range of 21 descriptors.

The comment from this group was that they were very impressed overall, by the total enhancement of character seen on the test cigarette.

The following table gives the order of ranking, within the sub-groups of related gum extracts, for each fraction:

S No.	Dose level g/100Kg	Rank Effect
S4	750	3
S1	750	1
S6	750	4
S8	750	2
S3	250, 750	-
S5	250	2
S2	250, 750	1
S7	750	3
S9	250, 750	4
S10	250, 750	4
S11	250, 750	1
S12	250, 750	2
S13	750	3

Rank order: 1 - highest 4 - lowest.

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The samples with highest ranking (1) within each group were considered to give enhancing notes compatible with the test blend.

The cigarettes were smoked 2 weeks after application of the gum extract.

B. B.A.T. GR & DC smoke assessment results:

The ad-hoc panel was used (7 members) to examine the effect in an open, paired-comparison type trial, using P162 standard cigarettes. An open questionnaire was used allowing expression of preference, characters detected and degree of compatibility of the extracts with the control tobacco blend. All samples (S1 - S13 and S14, 15, 18, 19, 22 and 23) were examined.

The following table gives the rank-order fractions showing positive, smoke-flavour enhancing effects: all samples were added at 300g/100Kg tobacco.

S No.	Rank effect
S4	2
S1	1
S6	3
S8	4
S3	(CBD) supportive effect on smoke
S5	1
S2	3
S7	2
S9	4
S10	2
S11	1
S12	3
S13	4

103472791

*This second table gives the numbers of samples which were identified as giving a significant effect in enhancing the smoke character.

<u>*S No.</u>	<u>General comments</u>
14	enhanced notes, green.
15	less irritant, fuller smoke
18	woody, increased green notes
19	lower pitch, enhanced woody
22	woody, green enhanced
23	slight chemical aftertaste, mellow earthy.

C. Overall conclusions from comparison of results

The most significant observation is that both groups, although using totally different methods of assessment, together with different control cigarettes, did show a high degree of correlation in what were considered to be the most enhanced smoke characters for given gum extract fractions.

From these results it can be inferred that the gum extracts that have been solvent partitioned with aqueous methanol, prior to chemical modification, do impart a more desirable and effective flavour enhancement. The trials in Southampton did select the samples from (S14 - 25) on this hypothesis. Thus the fraction mentioned above * were examined. Our hypothesis was, to a significant degree, proved by the smoke assessment of these fractions. The Malmö group agreed to examine these fractions on this basis. These results are to be communicated to us in due course.

3. GAS CHROMATOGRAPHIC ANALYSIS OF SAMPLES

The gas phase and semivolatile phase of the smoke from cigarettes treated with S1 and S2 have been compared with reference cigarettes.

There is no statistical difference between the 3 samples on gas phase analysis. Areas of the semivolatile phase results were deemed unsatisfactory and will be repeated but some results were available:

103472792

- a) The addition of S2 (0.25%) resulted in a 30% increase in solanone peak area.
- b) Although much greater amounts of norsolanadione than solanone were identified when the S2 fraction itself was analysed, no norsolanadione could be identified in the semivolatile phase. It may be that norsolanadione is chemically reduced during the smoking process.
- c) The region of the chromatogram between solanone and neophytadiene is considerably boosted by the presence of a range of norcembranoids.

This analysis will be repeated for S1 and S2, and S10 - S13 will be analysed. The technique (reported in the Journal of Chromatography about a year ago) involves steam distillation and continuous extraction. In order to simplify the complex chromatogram, it was decided to attempt to sub-fractionate the total sample.

Gc/exit port aroma assessment work has been carried out with S1 - S3 and S13. S10, S12 and S14 - S25 are yet to be evaluated.

The aroma of the constituents have been classified into the 9 character aroma profile:

	<u>S1</u>	<u>S2</u>	<u>S3</u>	<u>S13</u>
Green	x	x	x	x
Fruity	x	x	x	x
Floral	x	x	x	x
Woody	x			x
Aromatic	x	x	x	x
Phenolic	x	x	x	x
Earthy	x	x	x	x
Acidic	x	x	x	x
Spicey	x		x	x

The woody notes are present in S1 and S13 (derived from Palotina and Basma gums, respectively) but not in S2 and S3 (derived from McNair 944 gum and CBD, respectively). Thus the presence of a woody note is correlating

103472793

with the presence of labdanoid diterpenes in the gum extract. CBD did have a predominant earthy, phenolic region.

An attempt will be made to correlate these results with the results of smoke sensory work.

4. BIOLOGICAL TEST RESULTS

Biological testing has been transferred from the Karolinska Institute to the University of Stockholm, and Mr. B. Petterson was present from the University to describe recent results. Attached to this note are the results of toxicity testing (brown fat cell test and cell multiplication test) on the condensate from cigarettes treated with S11 and the results of mutagenicity testing (Ames test) on the condensate from cigarettes treated with S11. Also attached are extended Ames test results on condensate from cigarettes treated with S1, S2 and S3.

In every case, the results are negative, i.e. the condensate from cigarettes treated with S fractions is not more toxic or mutagenic than condensate from control cigarettes.

In view of the recent implementation of the Ames test in GR & DC, the technique adopted by Petterson was carefully questioned. Overall, it was recognised that the two techniques tallied closely, but two particular areas of difference emerged. GR & DC carry out at least 3 plates/test and at least 3 tests, whereas Stockholm conduct 1 plate/test over 3 tests. Also GR & DC work with fresh (same day) condensate extracts whereas Stockholm are prepared to allow the extract to age for several weeks prior to testing.

5. FUTURE TOBACCO GUM REQUIREMENTS

Fractions S14 - S25 inclusive are based upon Burley, Maryland and Virginia (Coker 319) gums generated in North Carolina in 1981. The results of CBD analysis of these gums were presented. Again, it was shown that the bulk of the CBD is obtained from the top half of the plant.

103472794

A review of the gums already available was given, together with an indication of the gums about to emerge from present experiments in Brazil. Through this source, the present stock of Virginia (MacNair 944 and Coker 319), Burley, Maryland, Basma and Palotina is about to be increased by further Virginia (Coker 347 and Coker 254), Burley, Palotina and Amarelinho samples.

A description of further leaf gum experimentation planned for the future was given and the Swedes were invited to comment.

The possible experiments described were:

a) Virginia Varieties:

NC95	-	high OBD
SC57	-	high mono-ols
SC58	-	high nicotine
NC744	-	very low CBD

b) Other tobacco sources of diterpenes:

N. Sylvestris	-	very high CBD
Bergerac PB	-	labdanoids only

c) Optimisation of diterpene yield:

A Virginia variety grown at 3 plant densities (all high) at various fertiliser regimes.

All three experiments were supported by the Swedes, but it was pointed out that we have not explored green leaf extracts of Oriental tobacco, a particularly rich source of flavour. Basma and Izmir might be examined, but it was acknowledged that appropriate growing conditions have to be found.

6. PLANNING OF NEXT STAGE

The next meeting has been preliminarily scheduled for 16th - 18th June, 1982, in Southampton.

In the intervening time interval, the following programme of sensory work will take place:

103472795

1. Malmö internal panel assessment of S14 - S25.
2. Southampton DELTA panel assessment of preferred fractions from S1 - S25 i.e. S1, S2, S5, S10, S11, S14, S18, S21, S23.
3. Ageing experiment using Southampton ad-hoc panel, intercomparing S5 and S2 fraction at 3, 8 and 12 weeks after manufacture.

Since it is apparent from the sensory work so far that samples based upon the aqueous methanol extract (both singlet oxygen oxidised and unoxidised) are preferred, this route will be explored in more detail chemically. It may be that an intermediate oxidation stage is desirable since, for example, S5 (unoxidised) may be too non-volatile in flavour terms, whilst S2 (exhaustively oxidised) may contain too much low molecular weight, highly volatile material. Experiments will be conducted at Stockholm which vary the conditions of the oxidative treatment:

1. Palotina and McNair 944 gums (aqueous methanol) subjected to mild singlet oxygen treatment and normal acid rearrangement. (2 samples)
2. The same gums subjected to strong acid rearrangement after strong singlet oxygen treatment. (2 samples)
3. The same gums subjected to strong singlet oxygen treatment but no acid rearrangement. (2 samples)

In addition a single sample will be generated from abienol subjected to strong singlet oxygen treatment and normal acid rearrangement. This will be the labdanoid equivalent of S3. In summary:

103472796

Sample	Treatment				
	Singlet Oxygen		Acid		
	Strong	Mild	Strong	Normal	None
1. Palotina (S1)	✓	✓		✓	
2. McNair 944 (S2)	✓	✓		✓	
3. Palotina (S1)	✓		✓	✓	
4. McNair 944 (S2)	✓		✓	✓	
5. Palotina (S1)	✓			✓	✓
6. McNair 944 (S2)	✓			✓	✓
7. Abienol (S3)	✓			✓	

(Control samples are shown in brackets)

In support of this work, further samples of Palotina, McNair 944 - and Coker 319 - gums will be provided to Stockholm. New samples deriving from this study will be added to the sensory assessment programme.

Gum samples at present being prepared in Souza Cruz will be analysed in Southampton and the results communicated to Stockholm. The chemical composition of these gums will determine to what extent there is a requirement to generate further samples from them.

At the meeting in June, in addition to a discussion of the results from the above, the following topics will be on the agenda:

1. Subfractioning of one or several of the best S samples in an endeavour to generate even stronger flavour samples.

103472797

2. Evaluation of S samples on ultra-low tar products.

3. Use of S samples in flavour compounding work.

and - most importantly -

4. Consideration of the commercial possibilities in the light of up-to-date sensory findings. If the picture still looks highly promising, it will be necessary to:

- i) Consider Patent protection
- ii) Negotiate a new Collaborative Agreement to cover the commercialisation phase.
- iii) Investigate the economics of the whole process.
- iv) Plan the steps to be taken to scale-up "green leaf extraction" and "extract fractionation/oxidation" plant.
- v) Conduct consumer-scale sensory assessment studies.

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103472798